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RESEARCHES ON FUNGI
VOLUME IV

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RESEARCHES ON FUNGI

VOLUME IV

FURTHER OBSERVATIONS ON THE COPRINI
TOGETHER WITH SOME INVESTIGATIONS ON
SOCIAL ORGANISATION AND SEX
IN THE HYMENOMYCETES

BY

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WITH FOUR PLATES AND ONE HUNDRED AND FORTY-NINE
FIGURES IN THE TEXT



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TO

MATHILDE BENSAUDE

WHO BY EXPERIMENTAL AND CYTOLOGICAL
INVESTIGATIONS ON A COPRINUS FIRST SHOWED
THAT HETEROTHALLISM OCCURS IN
THE HYMENOMYCETES

PREFACE

IN the second volume of this work, published in 1922, the Agari-cineae were divided into (1) the Aequi-hymeniiferae or Non-Coprinus Type of fruit-body organisation, made up of five Sub-types, and (2) the Inaequi-hymeniiferae or Coprinus Type, made up of six Sub-types. Of the eleven Sub-types the first was described in Volume II and eight more in Volume III. The two remaining Sub-types, the Curtus and the Plicatilis, are described in the first two chapters of the present volume. Thus a task proposed nine years ago has at length been brought to completion.

This volume is divided into two parts. Part I is devoted to the record of some further observations on the Coprini, while Part II treats of problems connected with social organisation and sex in the Hymenomycetes and particularly in the Coprini.

In a preliminary notice published in 1917, and in a more elaborate thesis published in 1918, Mlle Bensaude first demonstrated, by a combination of experimental and cytological methods, that the phenomenon of heterothallism occurs in the Hymenomycetes. The species upon which she worked was a Coprinus, and it was her stimulating papers that led to the experimental studies of sexual phenomena in the Hymenomycetes, especially in the Coprini, which have been carried out in my laboratory at Winnipeg. As some acknowledgment of Mlle Bensaude's pioneer work, this Volume has been dedicated to her.

In Part II, Chapter I, which treats of social organisation in the Higher Fungi, an attempt has been made to elucidate the physiological advantages which accrue to the mycelia through the existence of the numerous hyphal fusions which are so characteristic of them. Here, perhaps, new ground has been broken.

In the second and final chapter of Part II it is shown in detail that, in *Coprinus lagopus*, a diploid mycelium is able to diploidise an appropriate haploid mycelium. This discovery seems to provide a clue for the solution of the problem of the biological significance of conjugate nuclei. Incidentally, in the course of the work, it has been possible to calculate the speed of movement of nuclei derived from one haploid mycelium or an appropriate diploid mycelium along the hyphae of another haploid mycelium which the first mycelium is diploidising. In discussing the phenomena connected with the establishment of conjugate nuclei in mycelia which have been mated, it has been found convenient to employ the terms *diploidisation*, *the diploidisation process*, *to diploidise*, etc., first introduced by myself in an article recently published in *Nature*.

This volume contains one hundred and forty-nine illustrations in the text, including eighty-eight drawings and sixty-one photographs. Fourteen of the drawings have been borrowed from other authors. The other drawings were executed by my own hand or in conjunction with Miss Ruth Macrae. For copying the drawings reproduced in Figs. 85, 93, 94, and 95 my thanks are due to Dr. Nellie Carter. The source of each borrowed illustration is acknowledged in the text.

Of the sixty-one photographs in the text fifty-one were made under my direction, and the others were very kindly contributed by friends and correspondents: four by the late G. F. Atkinson; two by C. A. Pemberton; and one each by B. O. Dodge, Somerville Hastings, A. E. Peck, and the late J. E. Titley.

At the end of the volume have been appended four Plates which represent an attempt on the part of the author to visualise diagrammatically, in the present state of our knowledge, successive stages in the diploidisation (1) of one haploid mycelium by another haploid mycelium and (2) of a haploid mycelium by a diploid mycelium.

The researches recorded in this volume, together with other researches still to be published, were made the basis of a series of six lectures, called *Recent Advances in our Knowledge of the Fungi*, delivered under the auspices of the Norman Wait Harris Foundation in November, 1927, at Northwestern University, U.S.A.

My best thanks are due to the Canadian National Research

Council for grants in aid of the work. These grants have diminished my personal expense in publishing this volume and have enabled me to employ a research assistant, Miss Ruth Macrae, M.Sc. (McGill). Miss Macrae's help made it possible for me to carry out the long series of detailed experiments recorded in the last chapter of this volume, and I here desire to express my indebtedness to her for her valuable services. Once again, Mr. W. B. Grove, M.A., has been kind enough to give me the benefit of his help in reading the proofs.

A. H. REGINALD BULLER.

WINNIPEG, *January* 15, 1931.

TABLE OF CONTENTS

	PAGE
PREFACE	vii

PART I

FURTHER OBSERVATIONS ON THE COPRINI

CHAPTER I

THE CURTUS SUB-TYPE ILLUSTRATED BY *COPRINUS CURTUS*

Characters of the Curtus Sub-type—Representative Species— <i>Coprinus curtus</i> : Cultures—Fruit-bodies rendered Sterile by Fumes from Fresh Manure—Synonyms—Taxonomic Description—The Pilear Scales and the Pilocystidia—The Caulocystidia—The Pilear Flesh—The Gills—The Hymenium—The Giant Tramal Cells—The Spores—The Periodicity in Fruit-body Development—The Stipe—The Ripening of the Spores—The Opening of the Pileus—The Discharge of the Spores and the Autodigestion of the Gills—Concluding Remarks.	1
--	---

CHAPTER II

THE PLICATILIS SUB-TYPE ILLUSTRATED BY *COPRINUS PLICATILIS*

Characters of the Plicatilis Sub-type—Representative Species— <i>Coprinus plicatilis</i> —Geographical Distribution—Relations with its Substratum—Cultures—The Fruit-bodies—A Synonym: <i>Coprinus hemerobius</i> —The Pileus—The Gills—The Hymenium—The Spores—The Stipe—The Periodicity in Fruit-body Development and Time of Spore-discharge—The Ripening and Discharge of the Spores and the Absence of Autodigestion—The Generic Position of <i>Coprinus plicatilis</i> —The Absence of Autodigestion and its Significance— <i>Coprinus plicatilis</i> and <i>Coprinus longipes</i> —A Taxonomic Description of <i>Coprinus plicatilis</i>	33
---	----

CHAPTER III

VARIOUS REMARKS ON THE COPRINI

Comparative Remarks on the Coprinus and Non-Coprinus Types—Coprinus Ink—The Hyphal String in the Stipe of <i>Coprinus comatus</i> —A Further
--

TABLE OF CONTENTS

	PAGE
Remark on the Pileus-flesh of <i>Coprinus comatus</i> —A Giant Fruit-body of <i>Coprinus comatus</i> —Excretion of Drops of Water from a Pileus of <i>Coprinus niveus</i> — <i>Coprinus lagopus</i> growing on Beet Seeds	63

CHAPTER IV

THE GERMINATION OF THE SPORES OF COPRINUS STERQUILINUS AND OF
OTHER COPROPHILOUS COPRINI

<i>Coprinus sterquilinus</i> and its Spores—In Nature, Spores of <i>Coprinus sterquilinus</i> which Infect Horse Dung have all been previously Swallowed by Horses—Presence of Bacteria Not Required for the Germination of the Spores of <i>Coprinus sterquilinus</i> —High Temperature Not Required for Germination—Germination of Spores immediately after their Discharge—Possible Effect of Bacteria on Germination—The Struggle for the Substratum—Remarks on the Germination of the Spores of other Coprini	78
--	----

CHAPTER V

AN ANALYSIS OF THE FACTORS CONCERNED IN THE MECHANICAL FIXATION
OF THE FRUIT-BODY OF COPRINUS STERQUILINUS IN ITS SUBSTRATUM

Introduction—Fruit-body Fixation in certain Polyporaceae and Agaricineae— <i>Coprinus sterquilinus</i> —The Mycelium at the Surface of the Substratum—The Mycelial Strands within the Substratum—The Rudiments of Fruit-bodies—Light inhibits the Growth of Rudimentary Fruit-bodies—The Morphogenic Effect of Light on Developing Fruit-bodies—The Relations of the Fruit-body with Gravity—The Upward Pressure of the Stipe—The Heliotropic Effect of Light—The Mechanical Fixation of the Fruit-body—Conclusion	92
--	----

PART II

SOCIAL ORGANISATION AND SEX IN THE HYMENOMYCETES

CHAPTER I

SOCIAL ORGANISATION IN COPRINUS STERQUILINUS AND OTHER FUNGI

Social Organisation in Animals and Plants—The Algae—The Myxobacteriaceae—The Acrasieae—The Mycetozoa—The Phycomycetes—The Hymenomycetes—Hyphal Fusions and Clamp-connexions in the Hymenomycetes—Social Organisation in <i>Coprinus sterquilinus</i> —Social Organisation in Other Hymenomycetes—Social Organisation in Other Fungi—The Various Functions of Hyphal Fusions in the Hymenomycetes—Sex and Hyphal Fusions in the Hymenomycetes	139
--	-----

TABLE OF CONTENTS

xiii

CHAPTER II

	PAGE
THE EFFECT OF DIPLOID ON HAPLOID MYCELIA IN <i>COPRINUS LAGOPUS</i> AND THE BIOLOGICAL SIGNIFICANCE OF CONJUGATE NUCLEI IN THE HYMENOMYCETES AND OTHER HIGHER FUNGI	
Introduction—Definition of the Terms <i>Diploidisation</i> and <i>Diploidise</i> — <i>Coprinus lagopus</i> —Methods—Criteria of Sex—The Four Kinds of Haploid and the Two Kinds of Diploid Mycelia—The Pairing of Haploid and Diploid Mycelia—The Rate of Movement of Nuclei through a Haploid Mycelia which is being diploidised by (1) another Haploid Mycelium or by (2) a Diploid Mycelium—Direction taken by Nuclei in passing through a Haploid Mycelium which is becoming Diploidised—A Sex-factor Analysis of a Diploid Mycelium derived from a Large Haploid Mycelium which has been diploidised by a Small Diploid Inoculum—Observations on the Conversion of Haploid into Diploid Hyphae—The Number of Radial Hyphae of a Large Haploid Mycelium which are converted into Diploid Hyphae through the Action of a Suitable Haploid or Diploid Inoculum—The Frequency of Conjugate Nuclear Division in a Radial Diploid Hypha—Hyphal Fusions between All Possible Kinds of Mycelia—The Diploidisation of a Haploid Mycelium by a Theoretically Incompatible Diploid Mycelium—The Biological Significance of the Diploidisation of a Haploid Mycelium by a Diploid Mycelium—The Biological Significance of Conjugate Nuclei	187
GENERAL SUMMARY	295
EXPLANATION OF PLATES I-IV	307
PLATES I-IV	310
GENERAL INDEX	315

PART I

FURTHER OBSERVATIONS ON THE COPRINI

RESEARCHES ON FUNGI

CHAPTER I

THE CURTUS SUB-TYPE ILLUSTRATED BY COPRINUS CURTUS

Characters of the Curtus Sub-type—Representative Species—*Coprinus curtus*: Cultures—Fruit-bodies rendered Sterile by Fumes from Fresh Manure—Synonyms—Taxonomic Description—The Pilear Scales and the Pilocystidia—The Caulocystidia—The Pilear Flesh—The Gills—The Hymenium—The Giant Tramal Cells—The Spores—The Periodicity in Fruit-body Development—The Stipe—The Ripening of the Spores—The Opening of the Pileus—The Discharge of the Spores and the Autodigestion of the Gills—Concluding Remarks

Characters of the Curtus Sub-type.—The Curtus Sub-type of fruit-body possesses all the essential characters already described¹ for the Inaequi-hymeniiferous or Coprinus Type: (1) the gills are very thin, (2) the gills are parallel-sided, (3) the gills are not positively geotropic, (4) usually the hymenium on one side of a gill at maturity looks slightly downwards and that on the other side slightly upwards, (5) the spores ripen in succession from below upwards on each gill, (6) the spores are discharged in succession from below upwards on each gill, and (7) autodigestion proceeds from below upwards on each gill.

The special characters of the Curtus Sub-type which enable one to differentiate it from the other Coprinus Sub-types are as follows:

(1) The gills are parallel-sided and at first are connected together around the stipe by means of small flanges which run along their

¹ These *Researches*, vol. iii, 1924, pp. 118–119.

inner edges. Each flange is composed of two divergent flaps ; and each flap meets with, and is continuous with, a similar flap belonging to an adjoining gill. The flanges thus come to form a continuous cylinder around the stipe in the unexpanded fruit-body. In possessing this cylinder the Curtus Sub-type resembles the Comatus Sub-type, although in the former the flanges are much smaller and relatively simpler in construction than in the latter.

(2) Cystidia are entirely absent from the faces of the gills. In this respect the Curtus Sub-type resembles the Comatus Sub-type and differs from the other Coprinus Sub-types.

(3) The interlamellar spaces between adjacent gills, which are required to render possible the free development of the spores on the hymenium, are secured not by cystidia acting as stays or distance-pieces but, firstly, through the existence of flanges on the gill-edges, secondly, by an appropriate separation of the gills where they adjoin the pileus-flesh and, thirdly, by the gill-plates being very shallow and sufficiently rigid.

(4) The basidia are dimorphic. They are of two lengths, long and short. In this character there is an agreement with the Comatus, the Atramentarius, and the Lagopus Sub-types, but a difference in respect to the Micaceus and the Plicatilis Sub-types, the former having tetramorphic basidia and the latter dimorphic-trimorphic basidia.

(5) The fruit-bodies are very small and, at the moment when spore-discharge begins, rapidly open out like a parasol, so that the top of the pileus becomes flattened. In these respects the Curtus Sub-type differs very strikingly from the Comatus Sub-type.

(6) The pileus-flesh is extremely thin and, from the first, is divided into rays by grooves which are situated above the gills where these are attached to the flesh. As the pileus expands, these grooves open out and thus enable the pileus to change from the campanulate to the discoid form without being torn radially. In these respects the Curtus Sub-type resembles the Plicatilis Sub-type. Grooves, like those of the Curtus Sub-type, are also found in the Micaceus and the Lagopus Sub-types but are not present in the Comatus and the Atramentarius Sub-types.

(7) As the pileus opens out, the gills become cleft from above

downwards, so that in cross-section they resemble the letter Y. The gills are so shallow that the leg of the Y is very short.

(8) Autodigestion takes place from below upwards on each gill but only affects the lower unsplit part, so that the upper divided



FIG. 1.—*Coprinus curtus* (= *C. plicatiloides* of Vol. I). Fruit-bodies coming up spontaneously on unsterilised horse dung in a large glass chamber in the laboratory at Winnipeg at about 11 A.M. As each pileus flattens out, its disc becomes depressed like that of *C. plicatilis*. Natural size.

part of each gill and the extremely thin flesh remain intact. Relatively to the sizes of the fruit-bodies, therefore, there is much less autodigestion in the Curtus Sub-type than in the other Sub-types with the exception of the Plicatilis Sub-type where autodigestion does not take place at all.

In certain respects, namely, in possessing flanges, in the absence of cystidia, in the manner in which the interlamellar spaces are secured in the young fruit-body, and in the dimorphism of the

basidia, the Curtus Sub-type resembles very closely the Comatus Sub-type; but in its small size, in possessing grooves above the gills, in the flattening of the pileus before the process of spore-discharge begins, and in the very small amount of autodigestion, the Curtus Sub-type appears to stand very close to the Plicatilis Sub-type. The Curtus Sub-type seems to have the structure for spore-dispersal which one would expect to find in the Comatus Sub-type if this were to be very much decreased in size. The weight of very small gills is very much less than that of very large ones. In a small fruit-body, therefore, the flesh necessary to support and raise the gills can be very small in amount, and the raising of the gills can be accomplished by the little mass of flesh at the disc. In a massive fruit-body, such as that of *Coprinus comatus* or *C. atramentarius*, the flesh has more work to do, and its thickness must therefore be relatively greater than in a small fruit-body and its distribution above the gills must be more even.

Representative Species.—The only species with which I am acquainted that belongs to the Curtus Sub-type is *Coprinus curtus* Kalch.

Coprinus curtus: Cultures.—*Coprinus curtus* is a coprophilous species which produces small fruit-bodies gregariously at the surface of horse-dung balls in pastures. At Winnipeg, throughout the winter months, one may readily obtain cultures of the fungus from (1) fresh stable manure or (2) frozen dung-balls.

(1) Fresh horse-dung balls from a stable are placed in a large crystallising dish which is covered with a glass plate and then set in diffuse daylight on a laboratory table. At the end of ten days, almost invariably, fruit-bodies of *Coprinus curtus* begin to appear as tiny foxy-red rudiments scattered at the surface of the dung-balls; and, after about thirteen days, successive crops of fruit-bodies come to maturity daily for several days in succession (Fig. 1).

(2) Horse-dung balls dropped in the snow on the streets of Winnipeg during the winter become frozen solid in a few minutes. When such balls, which may have remained frozen for several weeks or even months, are taken into the laboratory and set in a covered crystallising dish, they soon thaw and, after about ten days, fruit-bodies of *Coprinus curtus* begin to appear at their surface in

the manner already described for dung-balls which have never been frozen.

The occurrence of *Coprinus curtus* fruit-bodies in cultures made in the manner just described may be explained as follows. Fruit-bodies of this fungus develop on horse dung lying on the prairie or in fields where horses graze. The spores are carried away by the wind and settle on grasses and other herbs to which they become so firmly attached that they are not dislodged either by wind or rain.¹ Hence, when hay is made, millions of the spores are adherent to the dry herbage. When a horse eats hay in a Winnipeg stable during the winter months, it swallows the spores with its fodder; and the spores, which have

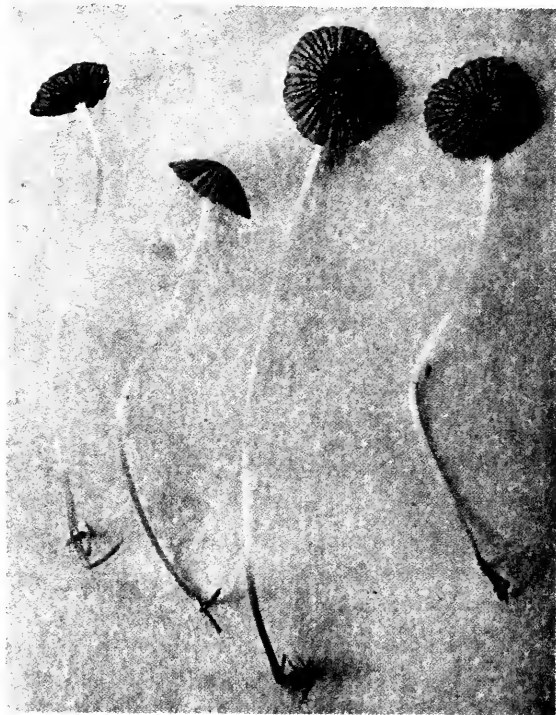


FIG. 2.—*Coprinus curtus*. Some fruit-bodies which came up spontaneously on dung-balls procured from England. About the maximum size for wild fruit-bodies (cf. fruit-bodies in Fig. 1). The two on the right shedding spores. The pilei are radially rimose, owing to the splitting of the gills down their median planes. In the expanded pilei the small discs are depressed. The pilear scales, which could be seen with a lens in the original photograph as very small colourless particles, are here practically invisible. Natural size.

retained their vitality in the dried condition, pass without injury down the alimentary canal of the animal concerned and, after completing the journey in from two to three days, come to be contained in the solid faeces dropped to the ground. When

¹ Cf. these *Researches*, vol. iii, 1924, pp. 229-230.

fresh horse-dung balls obtained from a stable are kept at room temperature, the spores at once germinate; and, within a fortnight, the mycelium produces fruit-bodies. When horse-dung balls fall on to the snow in the open during winter, they become subjected to a temperature often ranging for some weeks from 15° C. to - 35° C. That this degree of cold does not injure the spores is indicated by the fact that frozen dung-balls taken from the streets of Winnipeg eventually produce large numbers of *C. curtus* fruit-bodies.

Pure cultures of *Coprinus curtus* (Figs. 3-5) were made in the following manner. Some fresh horse-dung balls or pieces thereof were placed in one-inch-wide test-tubes plugged with cotton-wool or in small crystallising dishes covered with glass plates, and sterilisation was effected by setting the tubes and dishes in an autoclave and subjecting them to steam at a pressure of 15 lb. for one hour. Spores from a spore-deposit collected on a sterilised glass slide were then sown on the surface of the dung. After about ten days, at room temperature and in diffuse daylight, fruit-bodies began to appear at the surface of the dung.

The mycelium of *Coprinus curtus*, whether derived from a single spore or from a large number of spores sown together, as discovered by Miss Mounce¹ never produces any clamp-connections. Brunswik,² using the fruiting criterion, as a result of a limited number of matings of monosporous mycelia, found that *C. curtus* is heterothallic, and this conclusion has been confirmed by a long series of experiments carried out by Miss Dorothy Newton working under my direction at Winnipeg. Miss Newton's criteria were the production of oidia and the mode of branching after pairing monosporous mycelia. In a series of experiments, confirmed by repetition, she found that the monosporous mycelia were of four kinds, (*AB*), (*ab*), (*Ab*), and (*aB*), and hence concluded that individual strains of *C. curtus* are quadrisexual. The paired mycelia were

¹ Irene Mounce, "Homothallism and the Production of Fruit-bodies by Monosporous Mycelia in the Genus *Coprinus*," *Trans. Brit. Myc. Soc.*, vol. vii, 1921, pp. 210-211.

² H. Brunswik, "Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*." In K. Goebel's *Botanische Abhandlungen*, Jena, Heft V, 1924, p. 124.

subsequently allowed to fruit, but the production of perfect or of imperfect fruit-bodies was irregular and did not coincide with expectation either for bisexuality or quadrisexuality. Hence the



FIG. 3.—*Coprinus curtus*. Pure culture on horse-dung balls. Fruit-bodies about to expand. The scales on the pileus, which could be seen with a lens in the original photograph as small and colourless particles, are here invisible. Photographed at Cornell University by the late G. F. Atkinson, to whom the author sent the culture. Natural size.

criterion of fruiting had to be discarded. Brunswik,¹ relying on this criterion alone and basing his argument on a very limited number of matings, came to the conclusion that individual strains of *C. curtus* are of the two-scheme type or bisexual. Since the mycelium

¹ H. Brunswik, *loc cit.*, p. 125.

of *C. curtus* never produces clamp-connexions—the outward and visible sign in most Hymenomycetes of the paired condition of the



FIG. 4.—*Coprinus curtus*. The same pure culture as that shown in Fig. 3, but about an hour later. The fruit-bodies are rapidly expanding their pilei and are about to discharge their spores. The gills are splitting down their median planes, thus causing the pileus to become radially rimose. The scales on the pileus, which could be seen in the original photograph as small and colourless particles, are here invisible. Photographed at Cornell University by the late G. F. Atkinson, to whom the author sent the culture. Natural size.

nuclei in each cell—for a final decision whether individual strains of *C. curtus* are bisexual or quadrisexual we shall have to rely upon cytological methods which still remain to be applied to the problem.

The fruit-bodies shown in Figs. 3–6 were produced from a polysporous sowing on horse-dung balls which had been sterilised

by means of steam applied at atmospheric pressure for one hour. The spores, doubtless, were of diverse sex, so that after the spores germinated monosporous mycelia of opposite sex fused together and soon produced a diploid mycelium. After this mycelium had been growing through the dung-balls for about a week, the balls were sent to the late Professor G. F. Atkinson at Cornell University. The mycelium then fruited, and Professor Atkinson kindly sent me the photographs of the fruit-bodies here reproduced.

The fruit-bodies produced in polysporous cultures on fresh sterilised horse dung are usually, although perhaps not always, distinctly larger than those which come up spontaneously on unsterilised dung-balls. In this connexion the reader should compare Fig. 1 (p. 3), which shows wild fruit-bodies of about average size, with Figs. 3 (p. 7), 4 (p. 8), 5 (p. 10), and 8 (p. 14), which show cultivated fruit-bodies. The largest wild fruit-bodies so far met with are shown in Fig. 2 (p. 5).

Fruit-bodies rendered Sterile by Fumes from Fresh Manure.—In a large glass case (3 feet long, 1·5 feet wide, and 2 feet high), one-half of the floor was covered by horse-dung balls which were two or three weeks old and which had produced and were producing many normal fruit-bodies of *Coprinus curtus*. Into this chamber there was introduced a mass of new horse dung sufficient to cover the other half of the floor, and then the door of the case was shut tightly. Two days later a considerable number of *C. curtus* fruit-bodies came up on the old dung and expanded; but, instead of becoming grey with ripened spores, they all remained pale and were partially or wholly sterile. There can be but little doubt that this sterility was due to the effect of fumes given off by the fresh horse dung.¹

Synonyms.—*Coprinus curtus*, although a fairly common fungus, was not described by Fries, Berkeley, Stevenson, Massee, or Cooke and, for some years after becoming well acquainted with the fungus,

¹ I have also observed that, when the spores of *Pilobolus longipes* have been sown on a mass of fresh sterilised horse dung contained in a glass crystallising dish tightly closed by a glass plate, many of the fruit-bodies—which come up in an atmosphere containing gases derived from the dung—are abnormal in form and colour. Some of the sporangia fail to develop their black pigment and then the orange-coloured spores can be seen *en masse* through the colourless sporangium-wall.

I was unable to identify it with any known species. Therefore,



FIG. 5.—*Coprinus curtus*. The same pure culture as in Figs. 3 and 4, about 3 hours after the stage shown in Fig. 3 and about 2 hours after the stage shown in Fig. 4. Probably photographed about noon. The fruit-bodies in a group on the left are still shedding spores, while those in a group in the centre, which have revolute pilei, have entirely discharged their spores and are now exhausted. To the right and left of the bases of the stipes of the median fruit-body group, as well as in the front of the culture, are a few rudimentary fruit-bodies which, after 24 hours, will resemble the mature fruit-bodies already described. Photographed at Cornell University by the late G. F. Atkinson, to whom the author sent the culture. Natural size.

for convenience in reference when describing the reactions of its fruit-bodies to external stimuli in Volume I of these *Researches*,

I called it *Coprinus plicatiloides*.¹ In 1910 I sent a pure culture of the fungus (Figs. 3–5) to the late Professor G. F. Atkinson² of Cornell University who, after studying and photographing the fruit-bodies, reported that the species was closely related to *Coprinus curtus*, a coprophilous species described by Kalchbrenner³ from South Africa in 1881, from which it differed in its larger size and in having a villose stem. Subsequently Lange⁴ found *Coprinus curtus* in Denmark, and he described and illustrated the species in 1915 in his monograph of the genus *Coprinus*. The species which Lange describes as *C. curtus* is certainly my *C. plicatiloides*, and the name *C. plicatiloides* must therefore now be regarded as a mere synonym for *C. curtus*.

Taxonomic Description.—Kalchbrenner's description of *Coprinus curtus* was much too brief and fragmentary to be satisfactory to the systematist; and even Lange's description lacks certain details which are helpful in distinguishing this species from its fellows, e.g. the absence of cystidia from the sides of the gills and the shape of the pilocystidia. On this account I shall here repeat, with additions and slight emendations based on further study, my own description of the fungus published in 1920.⁵

COPRINUS CURTUS *Kalchbr.*

Kalchbrenner in *Fungi Macowaniani*, *Grevillea*, Vol. IX, 1880–1, p. 133.—Lange, in *Studies in the Agarics of Denmark*, II, *Coprinus*, *Dansk Bot. Ark.*, Bd. II, 1915, p. 45, Pl. I, Fig. *h*.—Buller in *Three New British Coprini*, *Trans. Brit. Myc. Soc.*, Vol. VI, 1920, pp. 364–365, also in his *Researches on Fungi*, Vol. IV, 1931, Chapter I. Synonym: *Coprinus plicatiloides* Buller in *Researches on Fungi*, Vol. I, Figs. 26, 27,

¹ These *Researches*, vol. i, 1909, p. 69.

² G. F. Atkinson, *in litt.*

³ C. Kalchbrenner, "Fungi Macowaniani," *Grevillea*, vol. ix, 1880–1881, p. 133. Kalchbrenner, in describing *Coprinus curtus*, gives the correct size of the wild fruit-bodies and mentions the distinctive reddish bran-like scales on the pileus (*primum sub lente rubiginoso-furfuraceo, dein pulverulento*), but otherwise his description is very incomplete. He does not describe the spores or the cells making up the pileus-scales, and he fails to note the presence of the characteristic capitate pilocystidia on the pileus, the caulocystidia on the stipe, and the absence of pleurocystidia from the gill-sides.

⁴ J. E. Lange, "Studies in the Agarics of Denmark," *Dansk Botanisk Arkiv*, Bind II, 1915, p. 45.

⁵ A. H. R. Buller, "Three New British Coprini," *Trans. Brit. Myc. Soc.*, vol. vi, 1920, pp. 364–365.

pp. 70, 71 ; Vol. II, Fig. 32, p. 96 ; Vol. IV, Figs. 1-20.—The photograph shown in Fig. 4, Taf. XIII in Bd. VIII (1902) of Cohn's *Beiträge zur Biologie der Pflanzen* and labelled (p. 344) *Coprinus ephemerus* Bull. (in R. Falck's *Kultur der Oidien bei den Basidiomyceten*) is certainly *C. curtus* and not *C. ephemerus*.

Pileus 3-8 mm. high when young, 5-15 mm. broad when expanded and flattened, foxy-red or rufescent to tan colour at first,



FIG. 6.—*Coprinus curtus*. Rudimentary fruit-bodies the day before their expansion. The outer surface of each pileus is rough with small red scales and numerous pilocystidia. Photographed at Cornell University by the late G. F. Atkinson, to whom the author sent the culture. Magnification, about 3.

becoming grey to dark grey, at first oval to cylindrical or elliptical, then expanded and flattened with a small, strongly depressed disc, splitting along the lines of the gills from above downwards and becoming plicate, bearing a certain number of minute scattered, flaky, separable, rufescent or whitish scales composed of globose or elliptical cells, often in chains, 12-30 μ in diameter, some brown and some colourless, the walls smooth and not ornamented with evenly arranged crystals of calcium oxalate, the pileus also villose or downy with many colourless hairs 70-140 \times 5-10 μ , tapering upwards but enlarged capitately at the apex where a minute drop of clear fluid growing to 40 μ in diameter is exuded under moist conditions. Stipe 2-8 cm. \times 1-2 mm., white, becoming stained with dull yellow, equal, finely villose, especially

below, owing to the presence of clavate hairs (caulocystidia) similar to those present on the pileus (pilocystidia), otherwise smooth, hollow. Gills grey, then black, each one at first attached to the stem by the margin for its entire length, then adnexed, and finally free, linear, narrow, in large fruit-bodies 4-8 \times 1-1.3 mm., in small fruit-

bodies proportionately smaller; margin just before autodigestion begins white, slightly divided so as to form a flange, and somewhat fimbriate. Flesh at disc brownish (vandyke), thin. Spores black in

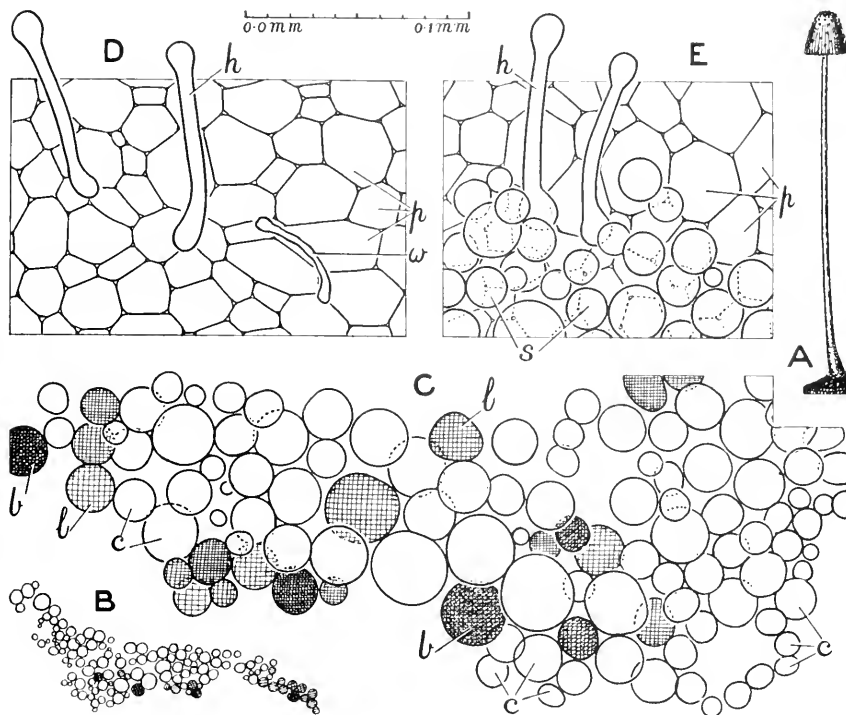


FIG. 7.—*Coprinus curtus*. A study of the surface of the pileus. A, a fruit-body on horse dung obtained from Kew, England, beginning to expand and exhibiting small reddish scales on its pileus. B, a pilear scale 0.7 mm. long, as seen in a surface view near the top of the pileus. C, about two-thirds of a pilear scale, as seen in a surface view of the pileus, showing: colourless cells *c*, light-brown cells *l*, and dark-brown cells *b*. D, a surface view of the pileus-flesh in a scaleless region, showing the palisade cells *p*, living capitate pilocystidia *h*, and a withered pilocystidium *w*. E, another surface view of the pileus-flesh showing the palisade cells *p*, scale-cells *s* above the palisade cells, and pilocystidia *h* which appear to have developed from scale-cells. A, natural size; B, magnified 64; C, D, and E, magnified 293.

the mass, dark brownish to black under the microscope, elliptical. $9-15 \times 6-9 \mu$. Cystidia (pleurocystidia) on the sides of the gills none. Clamp-connexions on the diploid mycelium always absent.

Habitat: on horse dung at Kew and Taunton, August and September, 1911; commonly coming up on horse dung in cultures

in glass dishes in England and central Canada, where daily crops of fruit-bodies open each morning. Occurs also in Germany, Austria, and South Africa.

The distinguishing characters of this species lie in the foxy-red colour of the very young pileus, the minute reddish or whitish scales which remain on the expanded pileus interspersed with clavate hairs, the finally depressed disc, the deep black spores, and the absence of cystidia on the sides of the gills. The pileus, when expanded, reminds one of that of *Coprinus plicatilis* but is much smaller. Sometimes very minute or dwarf fruit-bodies are to be found along with similar dwarfs of *C. lagopus* in crevices in old dung-masses.

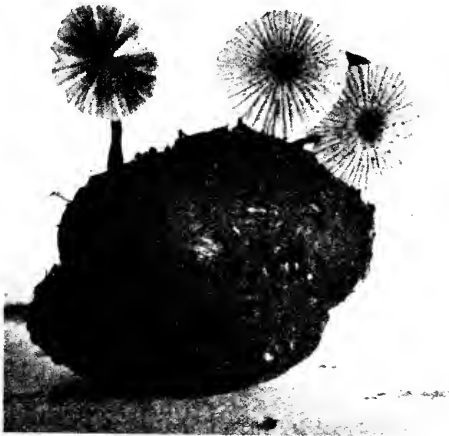


FIG. 8.—*Coprinus curtus*. Pure culture on a horse-dung ball, from spores of Winnipeg material. The ball has been turned to show the upper surfaces of the pilei. The discs are dark and depressed, and the small pilear scales, owing to being here of a very deep red colour, can be readily perceived. Natural size.

The fungus is common on horse-dung cultures at Birmingham, England, and at Winnipeg, Canada.

The Pilear Scales and the Pilocystidia.—In a tiny fruit-body rudiment in which

the pileus and the stipe have become clearly differentiated from one another, the pileus is covered externally with a thin, continuous, foxy-red or pale-red universal veil (Fig. 6). As the pileus grows in size, the veil breaks up into fragments; and it is these fragments which form the characteristic minute reddish or pale scales scattered over the pileus when this comes to maturity (Figs. 7, A, and 8). When a scale is examined microscopically, it is found to be composed of rounded or oval cells, 12–30 μ in diameter, some of which are colourless while others are more or less brown (Fig. 7, B, C, E). In scales which appear pale to the naked eye there are very few brown cells present, while in scales which appear

foxy-red to the naked eye the proportion of brown cells is high. The degree of redness in the pilear scales varies considerably in different fruit-bodies. Pale scales are very inconspicuous, and on account of their lack of colour and minute size cannot be distinguished with certainty in photographs, *e.g.* those reproduced in Figs. 2, 3, 4, and 5 (pp. 5, 7, 8, and 10). Deep red scales are at once apparent to the naked eye and, in a photograph, *e.g.* that shown in Fig. 8, they stand out as black dots. When a transverse section is taken through a pileus on the day before its expansion (Fig. 9), one observes that the brownest cells of each scale are the outermost ones and that all the inner scale-cells are colourless. The walls of a few of the very brownest and outermost cells are sometimes roughened by the presence upon them of a few irregularly placed crystals of calcium oxalate (Fig. 9, C, E, and F). However, in general, the walls of the scale-cells are quite smooth and they are never ornamented with minute evenly arranged calcium-oxalate crystals, so that in this respect they differ in a marked manner from the walls of the corresponding spherical cells which form the meal on the pilei of *Coprinus stercorarius* and *C. narcoticus*.

The pilocystidia, of which there are many hundreds on each pileus (Fig. 10), spring from cells which form part of the universal

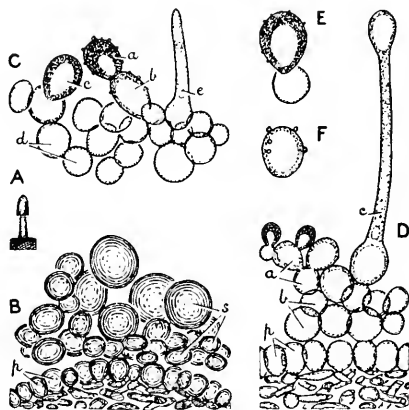


FIG. 9.—*Coprinus curtus*. Transverse sections through the reddish or white scales on the surface of the pileus. A, the fruit-body investigated. B, the scale *s* is lying over the palisade cells *p* above a gill. C, a scale showing contents of cells: *a* and *c*, cells containing dark-brown protoplasm; *b*, a yellowish cell; *d*, colourless cells; *a* and *b* each bear a few crystals of calcium oxalate on their exterior; *e*, a young hair growing apically, not yet capitate. D, a scale resting on the pileus-flesh: *a*, yellow and brown cells which have arisen by budding; *b*, colourless scale-cells; *c*, a full-grown capitate hair (pilocystidium); *p*, palisade cells of the subjacent pileus-flesh. E, a large, very brown scale-cell springing from a colourless cell, its protoplasmic contents are thick and dark-brown. F, a yellow scale-cell. Both E and F bear crystals of calcium-oxalate, some of which in F appeared to be in the vacuole. Magnification, 293.

veil, as shown in Figs. 7, E (p. 13) and 9, D, and therefore have the same mode of origin as the scale-cells. A single pilocystidium (Fig. 11) is a unicellular hair-like structure which has a somewhat



FIG. 10.—*Coprinus curtus*. A very young pileus and part of an unelongated stipe, photographed with a magnification of 32 diameters. To show the veil on the pileus breaking up into pilear scales, the pilocystidia projecting from the pileus, and some caulocystidia projecting from the stipe. Many of the cystidia bear at their tip a drop of mucilage.

swollen base, a tapering shaft, and a swollen capitate end. Its length varies from 70 to 140 μ and its breadth from 5 to 10 μ . The wall is thin and colourless, and it encloses colourless protoplasm containing one or more vacuoles (Fig. 11, *a*, *b*). When a pileus bearing fully grown pilocystidia is placed in air saturated with water-vapour, the end of each hair at once exudes a tiny drop of fluid which grows rapidly in size until it has attained a diameter of about 40 μ (Fig. 12). The drops thus formed exactly resemble those already described for *Psathyrella disseminata*.¹ Knoll² discovered that such drops, although soluble in water, persist in 95 per cent.

alcohol and are of a colloidal nature. The drops exuded by two or more hairs may come into contact with one another as they grow, with the result that they fuse and form one large drop (Fig. 12, *h* and *i*). When the drops dry up in dry air, their surface loses its

¹ These *Researches*, vol. iii, 1924, pp. 44, 46, Fig. 27, B.

² F. Knoll, "Untersuchungen über den Bau und die Function der Cystiden und verwandter Organe," *Jahrb. für wiss. Bot.*, Bd. L, 1912, pp. 463-464.

spherical shape and becomes irregular, thus revealing the colloidal substance which they contain (Fig. 12, c-f).¹

The Caulocystidia.—The caulocystidia resemble the pilocystidia in structure and function. Those at the base of the stipe are formed first and, as the stipe increases in length by intercalary growth just beneath the pileus, new caulocystidia are developed in acropetal succession. Thus the whole stipe becomes finely villose. A young fruit-body with an unexpanded pileus was placed in a compressor cell in air saturated with water-vapour. Immediately drops were

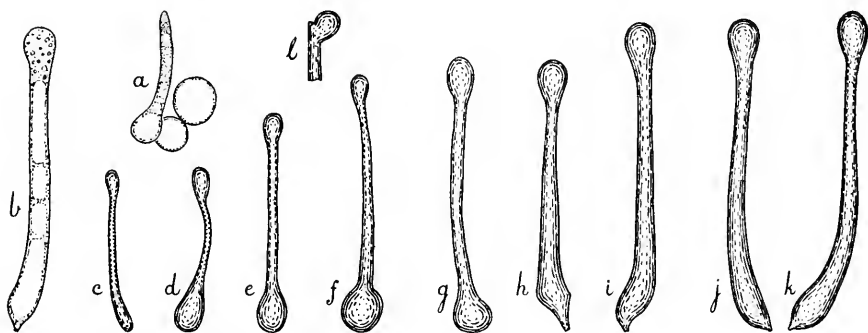


FIG. 11.—*Coprinus curtus*. Pileal hairs (pilocystidia): *a*, a young hair, half-grown, showing protoplasmic contents, as yet not capitate, accompanied by two scale-cells; *b*, a full-grown hair showing vacuolated contents; *c*–*k*, nine hairs showing variations in size and shape; all are capitate, the bases of some are rounded and of others pointed; *l*, the end of an abnormal hair which has lateral instead of a terminal capitulum. Magnification, 293.

excreted at the ends of the caulocystidia and pilocystidia. Then the fruit-body was exposed to dry air for some hours. As it dried, the drops slowly shrank and disappeared, leaving a film of mucilage behind, and the hairs became twisted. The fruit-body was then once more placed in air saturated with water-vapour. In the course of about half an hour a number of the caulocystidia recovered, and some of the drops grew to their former size. The stipe contained no water, and it therefore seems probable that the dried mucilaginous matter of the drops absorbed water from the air and then passed some of it into the shafts of the hairs. Knoll has supposed that the pilocystidia and caulocystidia act as hydathodes and assist

¹ The drops excreted by the sporangiophore of *Pilobolus* and by the hyphae of the mycelium of *Coprinus sterquilinus* also dry up with an irregular surface and are therefore also colloidal.

the fruit-body by excreting surplus water. Possibly, when a fruit-body is rather dry and suddenly becomes surrounded with air saturated with water-vapour, the hairs absorb water-vapour from the air and conduct it into the fruit-body.

The Pilear Flesh.—The flesh of the pileus is bounded externally by a palisade layer composed of rounded or pear-shaped cells which fit tightly together, and it is divided into strips by grooves (Fig. 16, A, p. 22) which run above the gills from the disc to the pileus-periphery. Owing to the presence of these grooves, a young pileus,

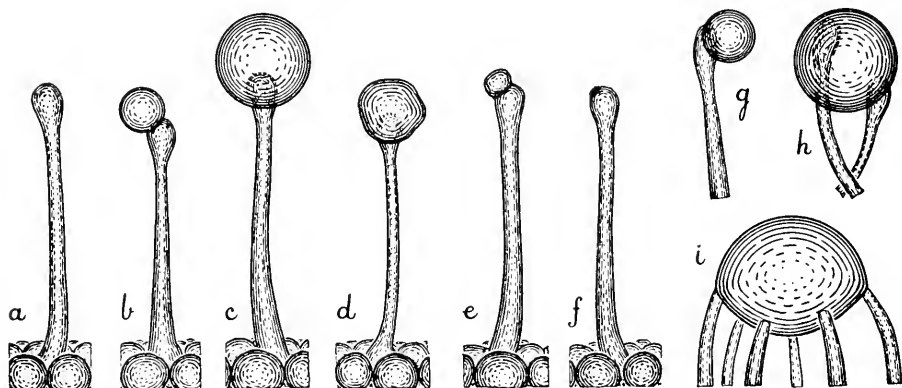


FIG. 12.—*Coprinus curtus*. Pilocystidia or pileal hairs. To illustrate their excretory function. Hairs: *a*, before excretion; *b*, a small drop has been excreted; *c*, a large drop has been excreted; *d*, the drop is drying up, its colloidal nature revealed by its irregular surface; *e* and *f*, further stages in the drying up of a drop; *g*, a single hair with a lateral drop; *h*, two hairs, with the two drops fused; *i*, six hairs, with their drops fused. Magnification, 293.

just before expansion (Figs. 3, p. 7, and 7, A, p. 13), appears to be longitudinally striate. Similar striae may be observed in the pileus-flesh of unexpanded fruit-bodies of *Coprinus plicatilis* (Fig. 24, p. 41) and *C. micaceus*. The grooves facilitate the opening of the pileus in a parasol-like manner (*cf.* Fig. 18, p. 27).

The Gills.—Fully developed gills in large fruit-bodies are 8–10 mm. long and 1–1.3 mm. wide in the middle (Fig. 13, A, B, and C); in small fruit-bodies they are proportionately smaller; while in dwarf fruit-bodies, the expanded pilei of which attain a diameter of only 2.5 mm. or even less, they do not exceed 1 mm. in length and a fraction of 1 mm. in width. In a young unexpanded fruit-body, the inner edges of the gills are all joined together; so that,

as in *Coprinus comatus* and *C. sterquilinus*, the stipe is completely surrounded with a white and sterile sheath. As the pileus opens, this sheath becomes split from below upwards along lines coincident with the interlamellar spaces. The two pieces of the sheath left attached to the free edge of each gill in an expanding pileus form a flange (Fig. 15, A and B, *m*) comparable with the flanges already described on the gills of *C. comatus* and *C. sterquilinus*. The thickness of each gill, measured between the outer walls of the paraphyses,

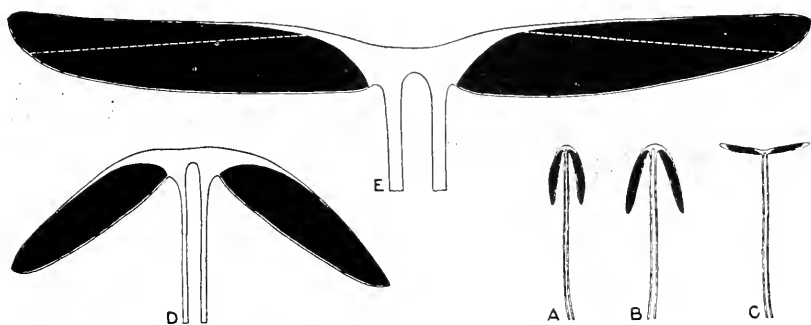


FIG. 13.—*Coprinus curtus*. Vertical sections through various fruit-bodies, A, B, and C, stages in the expansion of the pileus (the lower part of the stipe has been omitted): A and B, the spores are almost ripe; C, the pileus is turned slightly upwards and is shedding spores. D and E, stages in the expansion of the pileus on a larger scale: D, the pileus-flesh is growing radially below and is raising the gills; E, the pileus is now horizontally outstretched; the gills are free from the stipe and are about to begin to liberate their spores; the part of each gill below the broken line was in a vertical plane or almost so, while the part above the line was arched laterally (cf. Fig. 19). A, B, and C, natural size (large fruit-bodies); D and E (drawn with *camera-lucida*), magnified 6·8 times.

is only 0·05–0·08 mm. Few other agarics have such delicate gills.

The Hymenium.—The hymenium exactly resembles in structure that of *Coprinus comatus* and *C. sterquilinus*. It consists of a continuous sheet of paraphyses in which the basidia are set at intervals, long and short basidia alternating with one another. Pleurocystidia are absent (Figs. 14 and 15).

The gills of *Coprinus curtus* are so very narrow that it is difficult to cut them away from a pileus, lay them flat on a slide, and sketch their hymenium satisfactorily. Hence, in preparing the material from which the *camera-lucida* drawings shown in Fig. 14 were made, the following new method was adopted.

An expanding pileus, 5-6 mm. in diameter, was removed from the stipe of a wild fruit-body growing on horse dung in the laboratory, turned upside down on a glass slide, and covered with a cover-glass. The cover-glass was then pressed down lightly, with the result that there came into view, in a flattened and undamaged condition with the basidia and spores projecting upwards into air, all the radial strips of hymenium which had covered the highest parts of the interlamellar spaces. These pieces of hymenium originally were strongly arched, but the pressure of the cover-glass

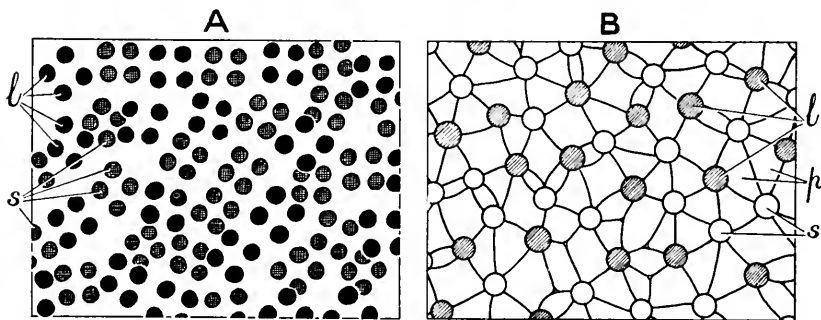


FIG. 14.—*Coprinus curtus*. Two surface views of the hymenium, made with the *camera-lucida*. A, showing the spores only: *l* (shaded black), spores of the long basidia; *s* (cross-hatched), spores of the short basidia. B, showing a plan of the basidia and paraphyses: *l*, long basidia; *s*, short basidia; *p*, paraphyses. Magnification, 293.

had flattened them out, thus making it possible to draw them with the *camera-lucida*.

The Giant Tramal Cells.—The trama is very thin. In the lower half of each gill in an expanding pileus it consists of a thin network of more or less cylindrical hyphae (Fig. 15, B, *t*); but, in the upper half, it is composed of two kinds of cells: (1) cylindrical hyphae, like those just described, which lie close beneath the hymenium on each side of the gill, and (2) spherical or oval cells which are scattered in the trama's central plane (Fig. 16, A). These spherical or oval cells, when the pileus is very young and before expansion has taken place, are quite small (Fig. 16, A, *d*); but, as the pileus expands, they swell up greatly in size, push apart the two sides of each gill, and thus assist in opening out the pileus like a parasol (Fig. 16, B and C). We may call these cells, since they greatly

exceed the other tramal cells in size, *giant tramal cells*. When a pileus is expanding, they are the largest cells in the whole fruit-body. The length and breadth of four of the largest were as follows : $60 \times 56 \mu$, $55 \times 55 \mu$, $62 \times 30 \mu$, and $60 \times 35 \mu$. The growth in size of the giant tramal cells may be realised by comparing Fig. 16, A, *d*, which shows them in a cross-section of a gill taken at 7.30 P.M. in the evening before the morning of pilear expansion, with (1) Fig. 16, B, *a*, which shows them in a similar section taken in the morning during the expansion of the pileus, and with (2) Fig. 16, C, *a* and *b*, which shows them in the sulcations of an expanded pileus, the pileus being viewed from above. Swollen giant tramal cells are also shown in the sulcations of the gills represented in Fig. 19 (p. 30).

The Spores.—The spores are black in the mass, dark brownish to black under the microscope, smooth, elliptical, $9-15 \times 6-9 \mu$ (Fig. 17). As may be seen by examining the hymenium in face view (Figs. 14, A, and 15, A), each spore, in reference to the axis of the basidium, is slightly broader

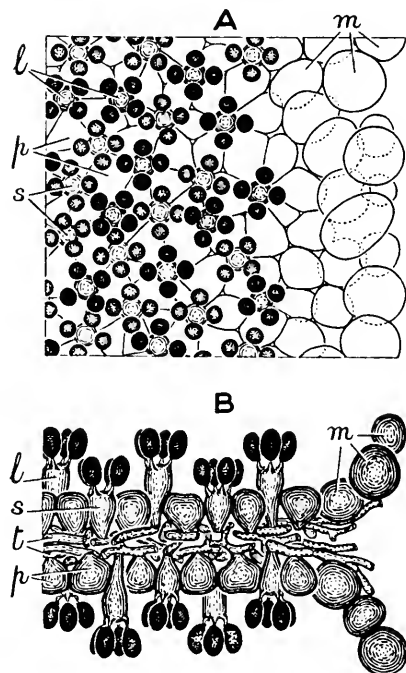


FIG. 15.—*Coprinus curtus*. The free edge of a gill bearing ripe spores, just before the beginning of the spore-discharge period. A, in surface view ; B, in cross-section : *l*, long basidia ; *s*, short basidia ; *p*, paraphyses ; *t*, the trama ; *m*, sterile cells at the extreme gill-edge. Magnification, 293.

in the tangential direction than in the radial. In this respect the spores of *Coprinus curtus* resemble those of *C. micaceus*,¹ *C. niveus*, and *C. plicatilis*,² but differ from those of *C. comatus*,³ *C. sterquilinus*,⁴ *C. atramentarius*,⁵ *C. lagopus*,⁶ and most of the other Coprini.

¹ These *Researches*, vol. iii, 1924, pp. 343-348, Figs. 153-156.

² *Vide infra*.

³ These *Researches*, vol. iii, p. 153, Fig. 60.

⁴ *Ibid.*, p. 202, Fig. 86.

⁵ *Ibid.*, p. 281, Fig. 120.

⁶ *Ibid.*, p. 321, Fig. 142.

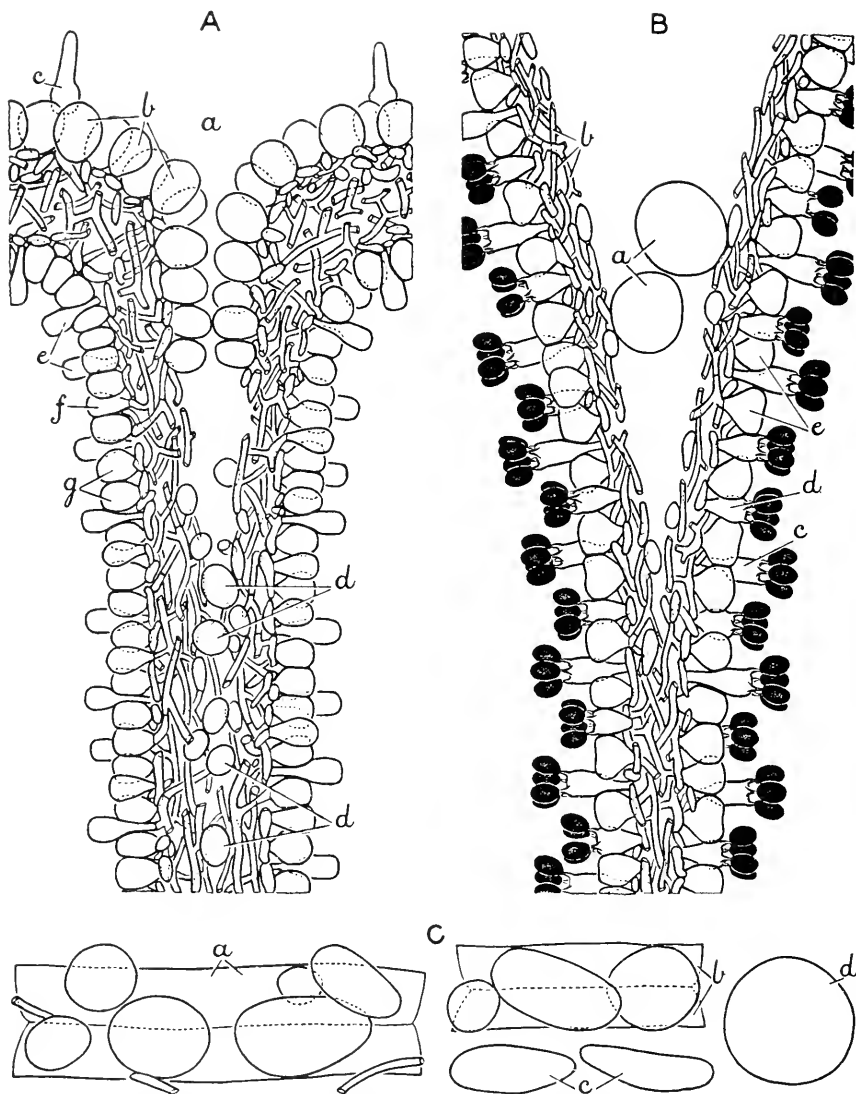


FIG. 16.—*Coprinus curtus*. To show splitting of a gill and the position and growth of giant tramal cells. A, transverse section of part of the pileus-flesh and upper part of a long gill at 7 P.M. on the day before pilear expansion: *a*, a groove in the flesh; *b*, palisade cells; *c*, a young hair not yet capitate; *d d*, giant tramal cells in the centre of the trama, at present relatively small; *e*, long basidia; *f*, a short basidium; *g*, paraphyses. B, transverse section of the middle part of a long gill in the morning of the day of spore-discharge when the pileus was expanding and the gill had become cleft down its middle plane: *a*, two giant tramal cells (drawn with the *camera-lucida*) now greatly swollen, in the cleft of the gill; *b*, ordinary tramal cells; *c*, a long basidium; *d*, a short basidium; *e*, two paraphyses. C: *a* and *b*, the bases of parts of two cleft gills seen from the top of the pileus (cf. Fig. 18) and outlined diagrammatically, containing greatly swollen giant tramal cells (drawn with the *camera-lucida*); *c*, two elongated giant tramal cells; *d*, the largest giant tramal cell observed. Winnipeg material. Magnification, 293.

The Periodicity in Fruit-body Development.—When fresh horse-dung balls are placed in closed crystallising dishes in the laboratory at Winnipeg, a crop of *Coprinus curtus* fruit-bodies usually appears upon it after about ten days; and, thereafter, new crops of fruit-bodies come to perfection each day for many days in succession. The expansion of the pileus culminating with the discharge of the spores—a process which occupies about an hour in dwarf fruit-bodies and three or four hours in very large

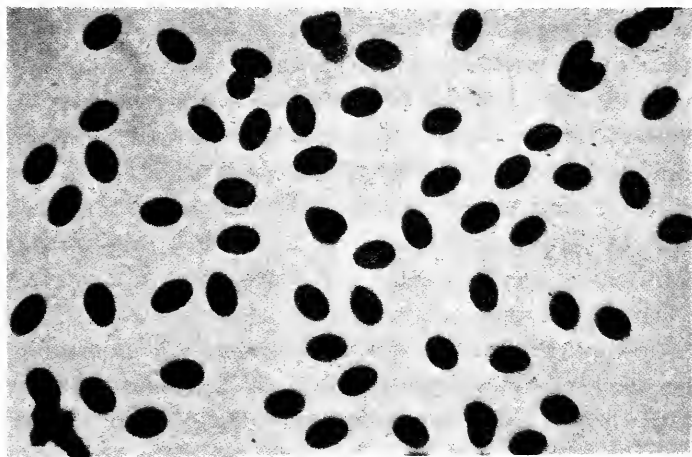


FIG. 17.—*Coprinus curtus*. Spores which fell on to a glass slide and were photographed dry. Their oval form and deep black appearance are well shown. Magnification, 500.

fruit-bodies (Figs. 3, 4, and 5, pp. 7, 8, and 10)—always takes place in the morning. In the afternoon an active culture contains : (1) collapsed fruit-bodies which shed their spores in the morning, and (2) rudimentary fruit-bodies at the surface of the dung, a few mm. high, which are destined to elongate their stipes during the night and to shed their spores the next morning. This periodicity in the development of fruit-bodies, as is shown by a series of experiments about to be recorded, is regulated by daylight.

One evening a culture of *Coprinus curtus*, like that just described, was placed in a dark-room. Next morning, in the dark, a new crop of fruit-bodies expanded and shed their spores in the usual way. This experiment, which was repeated subsequently, proves

that the last stages in the development of a fruit-body of *C. curtus*, which include the development of the spores, the elongation of the stipe, the expansion of the pileus, the discharge of the spores, and the autodigestion of the gills, can be carried through just as well in darkness as in the light.

The culture was kept in the dark. On the second day, under these conditions, another crop of fruit-bodies appeared on the dung ; but the stipes elongated only slightly instead of fully, while the pilei failed to develop their spores, remained foxy-red instead of turning grey, and did not expand.

On the morning of the third day in the dark, the crop of fruit-bodies just described, which under normal conditions of light would have opened on the second day, still remained imperfect. Their stipes had elongated somewhat more, but not fully ; while all the pilei were still foxy-red, sporeless, and unexpanded.

About 11 o'clock in the morning of the third day, the culture was taken out of the dark-room and placed by a window in strong diffuse daylight. In the afternoon, the crop of fruit-bodies which should have opened on the second day were still unchanged and, subsequently, they withered away without producing and shedding any spores. The light, however, was having a more favourable effect on the rudimentary fruit-bodies which were present in considerable numbers at the surface of the dung-balls.

In the morning of the fourth day, the culture having been exposed to daylight for 24 hours, more than one hundred new fruit-bodies came to perfection. Their stipes elongated, their pilei turned grey and expanded, and they shed their spores and underwent autodigestion in a perfectly normal manner. This was evidently due to the action of the daylight of the previous day on the rudimentary fruit-bodies which were then present at the surface of the dung.

From the series of observations just recorded we may draw the following conclusions. In *Coprinus curtus* daylight gives a morphogenic stimulus to the rudimentary fruit-bodies of such a kind as to enable them to elongate their stipes, ripen their spores, expand their pilei, and shed their spores. If fruit-bodies have previously received light daily, on the day of expansion they do not require any light

but will come to perfection and shed their spores in the dark. If, however, fruit-bodies have been deprived of light not only on the day they should shed their spores but also on the previous day, they are unable to elongate their stipes to the full extent, their pilei do not open, and no spores are developed.

So far as the periodicity in fruit-body development is concerned, the experiments point to the alternation of day and night as being the controlling factor. The final maturation of each fruit-body, as we have seen, is absolutely dependent on the action of light on the fruit-body when this is in a rudimentary stage of development, and we may conclude that it is the light on the day previous to pilear expansion, *i.e.* on the penultimate day, which provides the stimulus which enables a fruit-body to enter on its final development, a development which, when once started, can go on in the absence of light and which happens to attain its climax—the discharge of the spores—during the morning of the next day.

The Stipe.—The stipe, by means of intercalary growth in a region just beneath the pileus, begins to elongate during the afternoon of the day before the pileus expands and, at this time and until the next morning, it is positively heliotropic and ageotropic (Vol. I, p. 70, Fig. 26, D). At about 10 A.M. the next morning, just before the pileus begins to expand, the top of the stipe ceases to be positively heliotropic and becomes negatively geotropic, with the result that the pileus is turned gradually upwards until its central axis is in a vertical line (Vol. I, p. 70, Fig. 26, E). This response of the stipe to heliotropic and geotropic stimuli in succession results in the pileus being first pushed out from crevices between dung-balls into the open and then set in the best position for spore-discharge.¹

Just before a pileus is about to open, the top of the stipe is extremely sensitive to the stimulus of gravity. If one turns a fruit-body from a vertical to a horizontal position, the stipe begins to turn up the pileus within about three minutes after first receiving the geotropic stimulus.¹ Before the pileus comes to rest in a perfectly upright position, it may be swung by the stipe several

¹ These *Researches*, vol. i, 1909, pp. 69–70.

times past the vertical. An account of this phenomenon which was called *geotropic swinging* was given in Volume I.¹

The Ripening of the Spores.—The spores on the tiny gills of *Coprinus curtus*, just like those on the large gills of *C. comatus* and *C. atramentarius*, ripen on each gill in succession from below upwards. They are at first colourless; they then turn brown and finally black. At one stage in the development of the pileus there is a distinct gradation in the shade of each gill from black to brown from below upwards. This gradation in colour is less easy to detect than that which is so apparent on the maturing gills of *C. comatus* and *C. atramentarius*, but it is of precisely the same kind and has the same significance.

At 8 P.M. on the day before a pileus expands, the basidia and paraphyses can clearly be distinguished from one another in the hymenium, but the basidium-bodies of both the long and the short basidia have not as yet developed any sterigmata or spores (Fig. 16, A, p. 22). These come into existence during the ensuing night, and the spores ripen during the early hours of the next day. As the spores ripen, the increase in the amount of the dark pigment deposited in their walls causes the pileus as a whole to become grey. As soon as the spores are ripe, the pileus rapidly expands, thus permitting of their liberation.

The Opening of the Pileus.—The pilei of each diurnal crop of fruit-bodies open almost simultaneously during the morning hours. The process of spore-discharge is commenced as soon as the pileus has become flattened and, while spores are still being liberated, the pileus often becomes somewhat revolute. Photographs illustrating some successive stages in the expansion of the pileus are shown in Figs. 3–5. In Fig. 3 (p. 7) the stipes are still rapidly elongating at their apices, the pilei are still conico-cylindrical, and the spores on the gills are now black, thus giving the pilei their grey appearance. In Fig. 4 (p. 8), which shows the same fruit-bodies an hour later than Fig. 3, the stipes have now grown to nearly their full length, the pilei are rapidly expanding, the spores are ripe, and spore-discharge is about to begin. In Fig. 5 (p. 10), which shows the same fruit-bodies some three and a half hours later than Fig. 4,

¹ These *Researches*, vol. i, 1909, pp. 71–74, Figs. 27 and 28.

the pilei have now passed from the flattened (*cf.* Figs. 2 and 8, pp. 5 and 14) to the revolute condition, all the spores have been discharged from the gills so that these are now pale, and the stipes are about to collapse.

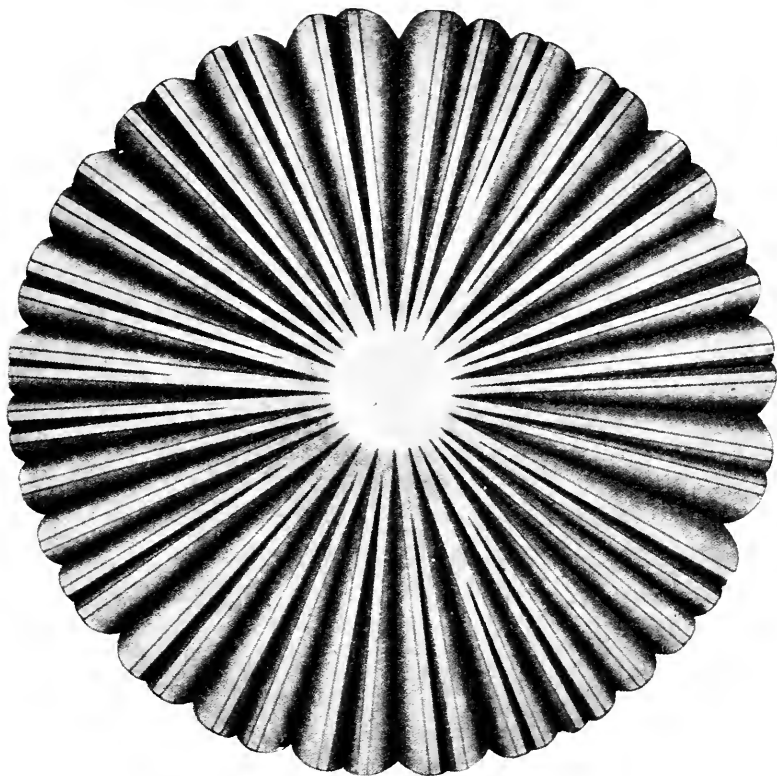


FIG. 18.—*Coprinus curtus*. Enlarged semi-diagrammatic drawing of the top of an expanded pileus, from which the scales and pilocystidia have been omitted, showing the central somewhat depressed disc, the pileus-flesh split up into radial ribbons, and the gills, all of which became cleft vertically down their median planes and thus allowed the pileus to open in an umbrella-like manner. The two halves of each gill have tramal cells above and hymenial cells below. Owing to the blackness of the spores and the transparency of the gill-halves, the split gills, in the view here shown, look dark-greyish or black. The original pileus was 21 mm. in diameter. Magnification, 5.

The pileus passes from the conico-cylindrical stage (Fig. 3), through the campanulate stage (Fig. 4) to the flattened stage (Figs. 2 and 8, pp. 5 and 14) in much the same manner as a parasol when it is opened. The opening of the pileus appears to be effected

in the main by growth in the size of the lower cells of the pileus-flesh surrounding the stipe at the disc. As soon as the spores are ripe, this tiny mass of flesh turns upwards with respect to the centre of the disc which thus becomes depressed and, in so doing, the flesh carries the gills with it, thus raising the gills into a horizontal position and flattening the whole pileus (Fig. 13, D, E, p. 19). The opening outwards of the pileus is doubtless aided by the swelling of the paraphyses around the upper parts of the interlamellar spaces. As this swelling continues, the top of the pileus as a whole gradually becomes concave (*cf.* Figs. 3 and 5, pp. 7 and 10). In *Coprinus curtus*, as in *C. sterquilinus* and all other Coprini, the paraphyses are the elastic elements of the hymenium and perform an important mechanical function as the pileus opens.

Owing to the presence of the radial grooves in the flesh, the looseness and weakness of the tramal hyphae, and the swelling of the giant tramal cells, each gill, when subjected to the tangential forces derived from the disc-flesh and the paraphyses, which tend to open out the pileus, splits radially from above downwards, thus becoming Y-shaped in a vertical transverse section. The radial sulcations thus produced in an expanded pileus can be readily seen in the photographs reproduced in Figs. 2 and 8 (pp. 5 and 14), and still more readily in the drawing of the upper side of a very large expanded pileus, shown five times the natural size without the scales and pilocystidia, reproduced in Fig. 18. In this figure it will be seen that the pileus-flesh, except at the disc, is split into radial ribbons and that each gill is partially cleft down its median plane into two halves (*cf.* Fig. 19). The split part of each gill, seen from above, turns from grey to dirty white as the spores are discharged.

The Discharge of the Spores and the Autodigestion of the Gills.—The discharge of the spores of *Coprinus curtus* begins immediately after the pileus has been opened and flattened like a parasol; and, as in all other Coprini, it proceeds from below upwards in each gill or, with respect to the pileus as a whole, centripetally (Fig. 13, C, p. 19). Just before the first spores to be discharged are shot away from their sterigmata, the basal part of the sterile flange of each gill is destroyed by autodigestion, so that it does not impede the

fall of the spores and so prevent them from escaping from the pileus. In this respect *C. curtus* resembles *C. comatus* and *C. sterquilinus*.

The hymenium of a gill which is discharging its spores (Fig. 19) exhibits, parallel to its gill-edge and from above downwards, five zones: (1) a zone of basidia bearing ripe spores; (2) a zone of basidia discharging spores, consisting of a higher sub-zone where only the long basidia are discharging spores and a lower sub-zone where only the short basidia are discharging spores; (3) a zone of basidia which have discharged their spores; (4) a zone in which the basidia and paraphyses are undergoing autodigestion; and (5) a zone of the products of autodigestion, situated along the gill-edge and containing the waste spores which failed to be discharged. These five zones resemble in their structure and physiology the similar series of zones on the gills of *Coprinus sterquilinus*.¹

During the discharge of the spores of *Coprinus curtus* the process of autodigestion affects only the sterile flanges and the lower unsplit portions of the gills, the upper V-shaped portions of the gills being left intact. These V-shaped portions of the gills, like the two sides of the positively geotropic wedge-shaped gills of the *Aequi-hymeniiferae*, always look downwards; and, on this account, from the point of view of the liberation of the spores, there would be no advantage in their destruction from below upwards.²

As the V-shaped parts of the gills liberate their spores, they turn from grey to white, and this change in colour proceeds from the periphery of the pileus toward the disc. After spore-discharge has begun, one can observe with the naked eye that the pileus, as a whole, consists of a white outer zone, which increases in width by centripetal advance, and a grey central zone which steadily diminishes in size and finally disappears. As the pilei of wild fruit-bodies of *Coprinus curtus* are so small, the zone of spore-discharge has only a few millimetres to travel. Hence, in this species, when contrasted with other Coprini having large gills, e.g. *C. comatus* and *C. atramentarius*, the duration of the spore-discharge period is very

¹ *Vide these Researches*, vol. iii, 1924, pp. 239-257.

² *Cf. these Researches*, vol. iii, pp. 119, 128, 291, Fig. 124.

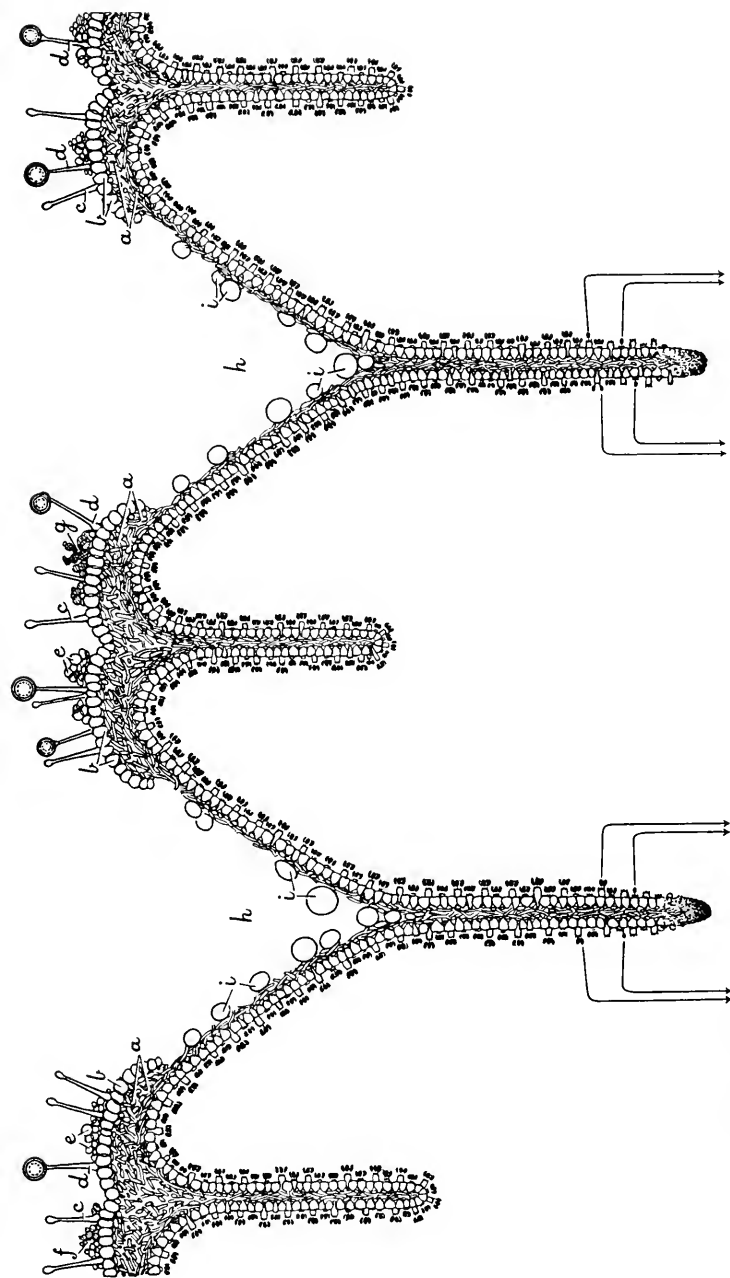


FIG. 19.—*Coprinus curtus*. Semi-diagrammatic vertical tangential section through an expanded pileus discharging spores, showing two long gills, three short gills, and three masses of pileus-flesh : *a a'*, three masses of pileus-flesh separated from one another owing to the expansion of the pileus which has involved the widening of the grooves in the flesh and the splitting of the long gills from above downwards ; *b b'*, the palisade cells of the pileus, projecting above which are numerous capitate hairs (pilocystidia) *c c'*, which, under moist conditions, excrete drops of a colloidal fluid as shown at *d d'* ; the scales of the pileus may be white as at *e*, brownish as at *f*, or dark-brown or black as at *g* ; *h h'*, sulcations due to the splitting of the long gills from above downwards during the expansion of the pileus ; *i i'*, giant trammel cells which, by swelling greatly, assisted in splitting the gills down their median planes ; the ordinary trammel cells are relatively thin and cylindrical and in the split portions of the long gills are exposed to view from the upper side of the pileus. The hymenium consists of a firm ground-layer of well-developed sterile paraphyses and of dimorphic (long and short) basidia bearing black spores ; cystidia are completely absent from the sides of the gills. The hymenial zones on the lower halves of the long gills are, as usual in *Coprinus*, from above downwards : (1) a zone with ripe spores, with the spores on all the basidia ; (2) a zone of spore-discharge, with two sub-zones—an upper for the long basidia and a lower for the short basidia—in which the trajectories of some of the spores when shot from their basidia in still air are indicated by arrows (each spore is shot horizontally about 0.1 mm.) ; (3) a zone of spore-free basidia ; (4) a zone of autodigestion and (5) at the free edge of each long gill, a zone of the products of autodigestion consisting of a watery fluid containing tiny particles and waste spores which were not shot away from their basidia. Winnupeg material. Magnification, 75.

short. Its actual length was found to range from about thirty minutes in very small fruit-bodies to about three and a half hours in very large fruit-bodies.¹

If an expanding pileus is taken from a fruit-body growing in a closed crystallising dish about 0·5–1 hour before spore-discharge would normally begin, and if the pileus is then turned upside down and placed in a closed compressor cell (*cf.* Fig. 103 in Vol. III, p. 240), spore-discharge commences almost at once and soon becomes very active. Preparations of this kind have often been used in the Winnipeg laboratory for the purpose of demonstrating spore-discharge to students. Upon looking down on an inverted pileus for a few minutes with the low power of the microscope, one may observe hundreds of spores as they are shot away from their sterigmata and fall through the air back on to the hymenium. The hastening of the beginning of the spore-discharge period may be due to the temporary exposure of the pileus to the relatively dry air of the laboratory.

Just before a spore is discharged from its sterigma, a drop of fluid is excreted in the same manner as in all other Hymenomycetes (Fig. 20, C), and it was in *Coprinus curtus* that I first saw the drop-excretion phenomenon in 1911. Several times, immediately after a spore had been shot upwards from a gill lying on a slide under

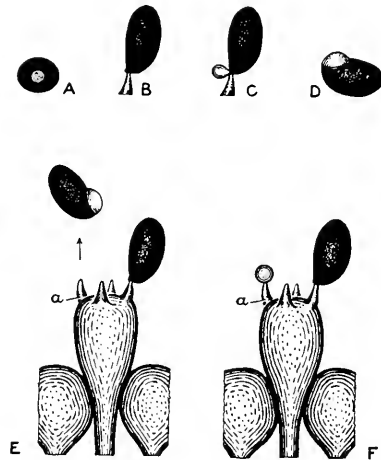


FIG. 20.—*Coprinus curtus*. Spores and their discharge. A, the top of a spore, to show the germ-pore. B, a spore on its sterigma, to show its projecting hilum just above the sterigma. C, a similar spore which has just excreted a drop of fluid from its hilum. D, a spore which was shot with its drop into the air from a gill and fell back on to the gill with the drop still attached. E, a basidium with two paraphyses; the sterigma *a* has just discharged its spore in the direction shown by the arrow; the drop is travelling with the spore. F, the same as E, but a few seconds later: the sterigma *a* has excreted a new drop. Usually no such drop is excreted. Magnification, 293.

¹ For a detailed account of the length of the spore-discharge period in *Coprinus curtus*, *vide* these *Researches*, vol. ii, 1922, p. 99.

a cover-glass and had fallen back on to the gill again, the drop was observed to be attached to the upper side of the spore (Fig. 20, D), thus proving that the spore and its drop had travelled through the air together. *Coprinus curtus* exhibits the same abnormalities in the discharge of the spores as those already described for *C. sterquilinus* and other Hymenomycetes.

It is the rule in Hymenomycetes that, when a spore has been discharged, the end of the sterigma concerned does not bear a drop. However, it was observed in *Coprinus curtus* that sometimes a sterigma, after shooting away its spore and the drop excreted at the spore-hilum, excreted at its apex a second drop of small size (Fig. 20, E and F) ; and the same phenomenon has been detected in *C. Rostrupianus*. This somewhat rare abnormality must be taken into consideration in any attempt to explain the spore-discharge mechanism.

Concluding remarks.—*Coprinus curtus* growing wild is one of the smallest of the Coprini, and yet a detailed examination of the fruit-bodies has shown that the mechanism for the production and liberation of its spores is truly inaequi-hymeniiferous and in many details resembles that of large species such as *C. comatus* and *C. sterquilinus*. It may be that the fruit-body of the original ancestral *Coprinus* which became evolved from one of the Aequi-hymeniiferæ far back in geological history was of medium size and that, relatively thereto, the fruit-bodies of *C. comatus*, *C. sterquilinus*, *C. atramentarius* and *C. picaceus* are very large, while those of *C. curtus* and *C. ephemerus* are very small.

CHAPTER II

THE PLICATILIS SUB-TYPE ILLUSTRATED BY COPRINUS PLICATILIS

Characters of the Plicatilis Sub-type—Representative Species—*Coprinus plicatilis*—Geographical Distribution—Relations with its Substratum—Cultures—The Fruit-bodies—A Synonym: *Coprinus hemerobius*—The Pileus—The Gills—The Hymenium—The Spores—The Stipe—The Periodicity in Fruit-body Development and Time of Spore-discharge—The Ripening and Discharge of the Spores and the Absence of Autodigestion—The Generic Position of *Coprinus plicatilis*—The Absence of Autodigestion and its Significance—*Coprinus plicatilis* and *Coprinus longipes*—A Taxonomic Description of *Coprinus plicatilis*

Characters of the Plicatilis Sub-type.—The Plicatilis Sub-type of fruit-body possesses all the essential characters already described for the Inaequi-hymeniiferous or Coprinus Type except one: (1) the gills are very thin, (2) the gills are parallel-sided, (3) the gills are not positively geotropic, (4) usually the hymenium on one side of a gill at maturity looks slightly downwards and that on the other side slightly upwards, (5) the spores ripen in succession from below upwards on each gill, (6) the spores are discharged in succession from below upwards on each gill, but (7), contrary to what we find in all the other Inaequi-hymeniiferous Sub-types, autodigestion does *not* proceed from below upwards on each gill.

The special characters of the Plicatilis Sub-type, which enable one to differentiate it from the other Coprinus Sub-types, are as follows:

(1) The gills are parallel-sided and do not possess flanges on their margins.

(2) Cystidia are present on the faces of the gills.

(3) The cystidia, in the unexpanded fruit-body, are not attached by both ends to opposing gills. Some of them bridge the interlamellar spaces, but others project freely into them as pegs. The

gills are shallow, and the interlamellar spaces required to provide room for the free development of the basidia and spores are secured : in part by the rigidity of the gills, in part by a suitable spacing of the gills where they adjoin the flesh and at their margins close to the stipe, and in part by the cystidia which act as guards and prevent two adjacent gills from anywhere touching one another with their hymenial surfaces.

(4) The cystidia do not connect adjacent gills during spore-discharge. When spore-discharge is about to begin, the pileus expands, with the result that adjacent gills become widely separated from one another. At this stage of development all the cystidia project from the gills as pegs.

(5) The pileus-flesh covering the gills is membranous and is provided with grooves which run radially above the longer gills. When the pileus expands just before and during spore-discharge, these grooves open out. As expansion of the pileus proceeds, the grooves are deepened so that the longer gills become split down their median planes for a certain distance. The result is that the expanded pileus, when seen from above, much resembles a parasol. Owing to the opening of the radial grooves and the splitting of the shallow gills for a certain distance down their median planes, the upper part of each long gill becomes widely V-shaped in cross section and each gill as a whole Y-shaped in cross-section. Owing to this change of form in the gills during the expansion of the pileus, a large part of the hymenium comes to look downwards towards the earth. This is distinctly of advantage in enabling the spores to escape from the fruit-body, and in a considerable measure it compensates for the lack of autodigestion of the gills. Spore-discharge commences only after the fruit-body has become expanded like a parasol.

(6) The basidia are irregularly dimorphic-trimorphic. The longest basidia are the most protuberant, the shortest practically non-protuberant, while the intermediate have an intermediate protuberancy. In the zone of spore-discharge there are from two to three sub-zones of spore-discharge corresponding to the irregular dimorphic-trimorphic grouping of the basidia.

(7) In an unexpanded fruit-body the pileus-flesh descends a

little way downwards so as to surround the upper part of the stipe with a short sheath, the lower margin of which is bounded by a collar made up of the inner ends of the gills. Shortly before spore-discharge begins, the sheath of flesh grows in such a manner that its rim moves outwards away from the stipe and upwards; and



FIG 21.—*Coprinus plicatilis*. A group of fruit-bodies coming up amid grass near London, England. The largest fruit-body is fully expanded: the disc is broad and characteristically depressed, and the radial sulcations are due to the partial splitting of the gills from above downwards along their median planes. Photographed by Somerville Hastings. Natural size.

it is this movement of the pileus-flesh which causes the expansion of the pileus as a whole.

(8) Autodigestion does not take place, so that the spore-freed portions of the gills are not destroyed from below upwards. This negative feature serves to distinguish the Plicatilis Sub-type from all the other *Coprinus* Sub-types.

Representative Species.—At present I am acquainted with only one species of fungus having the Plicatilis Sub-type organisation, and that is *Coprinus plicatilis* itself. This species, in possessing non-autodigesting (non-deliquescent) gills, appears to be unique so far as the genus *Coprinus* is concerned.

Coprinus plicatilis.—*Coprinus plicatilis* (Figs. 21 and 22, also Vol. III, Fig. 56, p. 138) is a small species of *Coprinus*, the fruit-bodies of which come up in a solitary manner on the ground among grass, etc., in many parts of the world. Its delicate parasol-like sporophores, which display themselves during damp weather in the summer and autumn, are well known to field-mycologists in England and other parts of Europe. The specific name *plicatilis* refers to the radial plications which mark the expanded pileus when seen from above. Owing to the depression of the disc at maturity and the very regular plications above the gills, a fully expanded pileus, when seen from above, is somewhat like a tiny wheel.

Geographical Distribution.—The occurrence of *Coprinus plicatilis* has been reported ¹: in Russia, Finland, Hungary, Italy, Germany, Denmark, Holland, Belgium, France, and Britain; in Japan, India, and Ceylon; in the United States and Behring Strait; in New Zealand, and in Queensland, Australia; so that this species evidently has a very wide geographical distribution. I am familiar with the fungus as it grows in England; Dr. Bisby and I ² have frequently met with it on the ground in woods near Winnipeg; and I have observed it in a grassy place at Kenora on the Lake of the Woods in central Canada.

Relations with its Substratum.—On a lawn in my father's garden at King's Heath, England, I saw fruit-bodies coming up every summer and autumn for about ten years in succession. A photograph of some of these fruit-bodies is reproduced in Fig. 22. The lawn in question was kept well mown and did not receive any manure; nor were there any roots of trees or sticks in the turf. Yet, during damp weather, a few fruit-bodies came up in different places on the lawn day after day. It seems clear, therefore, that the mycelium must have been growing upon the dead roots or rhizomes of grasses or upon corresponding structures of other herbs present in the turf along with the grass. I tried to trace the mycelium from the base of the stipe into some particular piece of

¹ G. Masee, "A Revision of the genus *Coprinus*," *Ann. of Bot.*, vol. x, 1896, p. 177.

² G. R. Bisby, A. H. R. Buller, and J. Dearness, *The Fungi of Manitoba*, London, 1929, p. 117.

the turf, but did not succeed. Since the fungus has never been reported as growing on wood or dung but only in more or less grassy places, and in view of my own observations just described, it seems that *Coprinus plicatilis* under natural conditions is neither coprophilous nor lignicolous, but is graminicolous. The exact relations of the mycelium to the turf in which it flourishes, however, require further investigation.

Cultures.—Some expanding fruit-bodies of *Coprinus plicatilis*



FIG. 22.—*Coprinus plicatilis*. A series of fruit-bodies on turf removed from a lawn. The large one in the fore-ground with the depressed disc is fully expanded. Photographed at Birmingham, England. Natural size.

were collected one morning early in September from a damp lawn in Kew Gardens. The pilei were at once removed from their stipes and set over glass slides in boxes, with the result that, in the course of an hour or two, several dense black spore-deposits were obtained. These deposits were conveyed to Winnipeg and, a few months later, at my request, Mr. T. C. Vanterpool, a research worker, kindly took charge of them and sowed some of the spores on dung-agar. Germination took place, and the mycelium, after growing a few days on an agar plate, was transferred to wide tubes of sterilised horse dung. The mycelium, under these conditions, grew slowly and, after three months, produced a number of rudimentary fruit-bodies ; but these showed no vigour, for they failed to grow to full size and

they neither elongated their stipes nor expanded their pilei. Thus the attempt to cultivate *C. plicatilis* on horse dung failed. Under similar conditions of culture *C. lagopus* and *C. sterquilinus*—both of which occur on horse dung in pastures—never fail to fruit well, the former in about 14 days and the latter in about 28 days after inoculation of the dung with spores. The failure of *C. plicatilis* to flourish in artificial dung-cultures helps to explain why this species is never found on horse dung under natural conditions in the open. Possibly it might be grown successfully on sterilised grass-plants.

The Fruit-bodies.—Some fruit-bodies in various stages of development obtained from an old lawn at Birmingham, England, are shown in Fig. 22. They were photographed immediately after being removed from the lawn and therefore exhibit their natural forms. The two fruit-bodies shown in Fig. 21 (p. 35) were coming up amid grass near London, England. Two other fruit-bodies, shown in Vol. III, Fig. 56 (p. 138), were photographed as they grew beneath some trees in Queen's Cottage Grounds, Kew Gardens.

A Synonym: *Coprinus hemerobius*.—In *Coprinus plicatilis* the expanded pileus is 1·3–2·5 cm. in diameter and the stipe 3–9 cm. high; but exceptional fruit-bodies with the pileus 4 cm. in diameter and the stipe 6–9 cm. high are sometimes found. These exceptional fruit-bodies, with pilei of about twice the ordinary size, may seem, at first sight, to belong to another species; and I am inclined to believe that *Coprinus hemerobius* is nothing more than a large *C. plicatilis*.

Once at Kew, in the grounds of Queen's Cottage, I found some exceptionally large expanding fruit-bodies of *C. plicatilis* which at first I took to belong to *C. hemerobius* (Fig. 23); but a careful examination showed that they agreed with ordinary *C. plicatilis* fruit-bodies in: the characters of the stipe (A), the large date-brown disc (A, *a* and *b*), the nature of the grooves in the pileus-flesh (A, *b*), the palisade cells of the flesh (B, *a*), the trimorphism of the basidia, the nature and distribution of the cystidia (B, *b* and *c*), the blackness and large size of the spores (B, *f*), and in the spores having three differing dimensions (B, *d* and *e*). Both the spores and the cystidia (B, *c-f*), like the unexpanded pileus (A, *b*), were

longer than usual; but only in one point did there seem to be a difference which might be important, namely, in the spores being

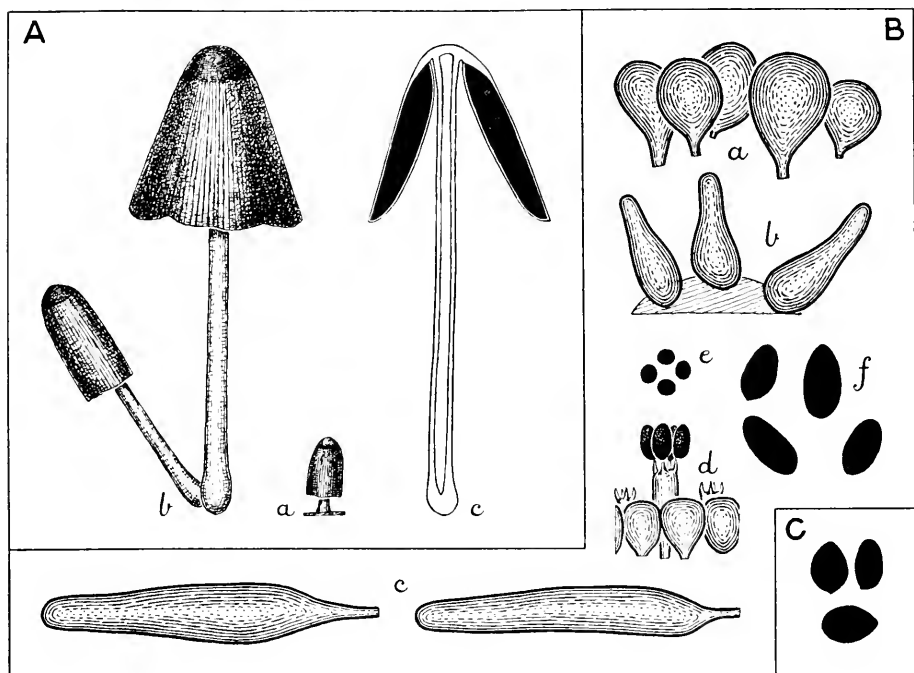


FIG. 23.—An extremely large form of *Coprinus plicatilis*, probably identical with the *C. hemerobius* of earlier authors, obtained in Queen's Cottage Grounds, Kew Gardens. The pileus is unusually large and the spores and cystidia unusually elongated. A: a, a very young fruit-body; b, two older fruit-bodies, the larger one expanding; c, a vortical section through the larger fruit-body shown at b. B, details of structure of the larger fruit-body shown at A b: a, palisade cells from the upper surface of the fruit-body; b cystidia (cheilocystidia) on the edge of a short gill; c, two cystidia (pleurocystidia) which projected from the side of a gill; d, a piece of the hymenium showing sterile paraphyses, one long basidium bearing spores, and two short basidia on which the spores have not been represented; e, four spores of a basidium seen from above, showing two differing dimensions for each spore; and f, some of the elongated spores, lying in water. C, spores of a normal *C. plicatilis* fruit-body for comparison with those shown at B, f. Magnification: A, natural size; B, a-e, 293; B, f, 683; C, 683.

compressed-oval although somewhat pointed at the apex (B, f), instead of being distinctly flattened heart-shaped (cf. B, f and C). However, the spores in different normal *C. plicatilis* fruit-bodies vary much in size and shape (Fig. 32, p. 48). I therefore came to the conclusion that the fruit-bodies under discussion, notwithstanding

that they were so large and that their spores were not typically heart-shaped, ought to be included in *C. plicatilis*.

Although for many years I have paid particular attention to the genus *Coprinus* and have examined upwards of thirty of its species, *Coprinus hemerobius* is still unknown to me as a distinct species. That *C. hemerobius* is only a form of *C. plicatilis* is a view also expressed by Lange.¹

The Pileus.—The pileus of *Coprinus plicatilis* at first is greyish-brown and ovate, then campanulate, and finally almost plane, although still convex. Fine striae run from the margin of the unexpanded pileus to the large disc, which is at first somewhat umbonate but later becomes distinctly depressed (*cf.* Figs. 21 and 22). They mark the position of furrows in the flesh and are situated, as in *C. micaceus* and *C. curtus*, above the lines of attachment of the gills (Fig. 24). As the pileus expands, these furrows open out, the upper parts of the gills become split into two halves from above downwards, and the top of the pileus becomes rimoso-sulcate as in *C. curtus* (*cf.* Fig. 18, p. 27, and Fig. 22, p. 37). This parasol-like mode of opening permits of the pileus being fully expanded without being torn into rays. The pileus, unlike that of *C. curtus*, never becomes quite plane and then more or less revolute; but, when fully expanded, it is always convex (Figs. 21, 22, and 34, pp. 35, 37, and 51). The expanded pileus, as already pointed out, is 1·3–2·5 cm. in diameter, varying up to 4 cm. The depressed disc, which retains its brown colour when the rest of the pileus has turned pale after the loss of the spores, is usually 4–5 mm. in diameter.

The flesh of the pileus is relatively thick at the disc; but, where it covers the gills, it is extremely thin and divided into rays by the furrows or sulcations already mentioned. The disc-flesh, in the unexpanded pileus, is prolonged downwards 0·5–1·0 mm. in such a way as to surround the stipe with a closely fitting sheath terminated below by a collar formed by the inner edges of the gills (Fig. 25, A).

The expansion of the pileus (Fig. 25) is accomplished by the growth of the disc-flesh where this ensheathes the top of the stipe

¹ J. E. Lange, "Studies in the Agarics of Denmark," *Dansk Botanisk Arkiv*, 1915, Part II, *Coprinus*, p. 49.

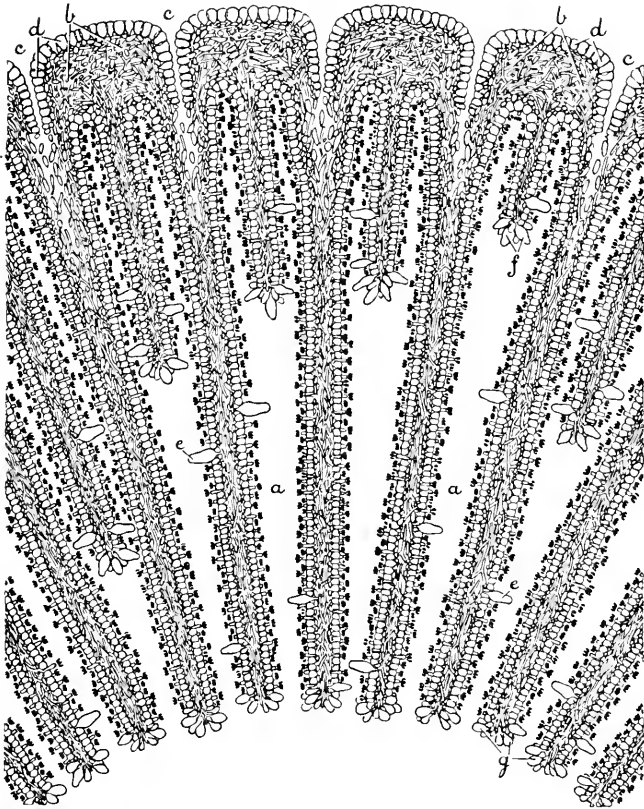


FIG. 24.—*Coprinus plicatilis*. Semi-diagrammatic transverse section (taken in a horizontal plane) through an expanding pileus (cf. A in Fig. 25). The inner edges of the long gills have already been pulled away from the stipe and the interlamellar spaces *a a* are widening as the pileus expands. The pileus-flesh *b b* is divided by grooves *c c* which lead down to loose tramal cells of the long gills; by splitting down these planes of weakness, the pileus is able to expand and become plicate. The pileus-flesh is bounded on its outer side by the palisade cells *d d*. Attached to the pileus-flesh are the long gills and the short gills which alternate with one another. The hymenium is made up of conspicuous sterile paraphyses (a sheet of connected cells), trimeric basidia bearing black spores, and cystidia *e e*. The basidia project freely into the interlamellar spaces. The cystidia on the sides of the gills (pleurocystidia) *e e* appear to act as guards in that they prevent opposing hymenia pressing against one another during the development of the spores. There are also cystidia (cheilocystidia) *f* on the ends of the free edges of the short gills and swollen cells *g* on the edges of the long gills. English material. Magnification, 42.

(A). This lower part of the disc-flesh grows both radially and tangentially, whereas the upper part of the disc-flesh does not. Owing to this differential mode of growth of the disc-flesh (*cf.* A, B, and C), the collar at the top of the gills (D, *c*) is gradually turned outwards and upwards, the gills leave the stipe and become turned through a right angle into horizontal positions, a naked zone of bare flesh 0.5–2.0 mm. in width (D, *b*) comes to surround the stipe



FIG. 25.—*Coprinus plicatilis*. Vertical sections showing three stages in the development of a fruit-body, drawn semi-diagrammatically, to show how the growth of the pileus-flesh brings about the expansion of the pileus. A : the gills are vertically disposed, and they are black owing to the presence of ripened spores; the pileus is slightly unbonate; the pileus-flesh is about to raise the gills. B : the under part of the pileus-flesh between the gills and the stipe is growing radially and is thus expanding the pileus. C : the under part of the pileus-flesh between the gills and the stipe has now grown radially to its maximum extent and has thereby completely expanded the pileus; the disc is now depressed; the inner ends of the gills are widely separated from the stipe; and spore-discharge is about to begin. D : view of the middle part of the expanded pileus of C seen from below; *a*, the stipe; *b*, a bare area of pileus-flesh between the gills and the stipe; and *c*, a collar formed by the union of the inner ends of the gills. The change from A to C took place in a single morning. Enlarged to 1.33 the natural size.

below and to separate the stipe from the gill-collar, and the disc of the pileus, as viewed from above, becomes depressed (C).

Marasmius rotula (Fig. 26), another small agaric, found on sticks, resembles *Coprinus plicatilis* in its general form. It, too, has a sheath of flesh surrounding the top of the stipe in the unexpanded pileus, a very decided gill-collar, a pileus which is convex when expanded, and a disc which becomes depressed. The expansion of the pileus in this species appears to take place in the same manner as that just described for *Coprinus plicatilis*.

The pileus of *Coprinus plicatilis*, when just fully expanded and seen from above, is date-brown at the depressed disc and elsewhere greyish or cinnereous, owing to the fact that the black spores are seen collectively through the translucent flesh and through the

sulcations above the gills. However, as the spores are shed, it becomes much lighter and finally whitish, except at the disc which remains brown.

The upper surface of the pileus is made up of palisade cells. These cells in some fruit-bodies are more or less pear-shaped and in others oval or even almost spherical (Fig. 27). They are firmly attached to one another laterally, and there is an intercellular space wherever three meet together.

The pileus of *Coprinus plicatilis* at maturity is smooth, but in



FIG. 26.—*Marasmius rotula*, one of the acquihymeniiferous Agaricineae, which vegetates in dead twigs, sticks, and roots in woods and hedgerows. Each pileus has its gills, like those of *Coprinus plicatilis*, adnate to a collar free from the stipe. Photographed at Scarborough, England, by A. E. Peck. Natural size.

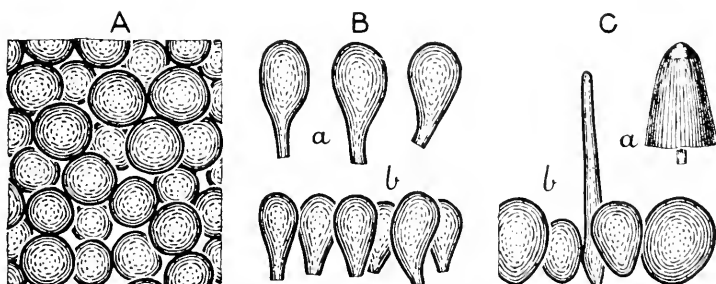


FIG. 27.—*Coprinus plicatilis*. Palisade cells on the outer surface of the pileus-flesh (cf. Fig. 24). A, surface view; fruit-body obtained at Kew, England. B: a, a lateral view of three of the cells shown in A; b, palisade cells of a fruit-body obtained on a lawn at King's Heath, England. C: a, a fruit-body, obtained from a field at King's Heath; b, some palisade cells from the fruit-body a. Hairs are usually absent from the pilei of *C. plicatilis*, but a few were found on that of a and one is shown in b. Magnification: A, B, and C b, 293; C a, natural size.

some young unexpanded fruit-bodies I once observed a few pilocystidia, one of which is shown in Fig. 27, C.¹

¹ The expanded pileus of *C. micaceus* is devoid of pilocystidia; but, in this species also, I have observed a few of these hairs in some young unexpanded pilei.

The Gills.—The gills are usually 6–10 mm. long, but varying in the largest fruit-bodies up to 17 mm. long; 1.5–3.0 mm. broad; parallel-sided, except near the furrows in the flesh where they are somewhat expanded (Fig. 24, p. 41); and in their unexpanded part 0.1–0.15 mm. thick, the thickness being measured between the outer walls of the two layers of paraphyses. While they resemble the gills of *C. curtus* in their mode of splitting from above downwards, they differ from them

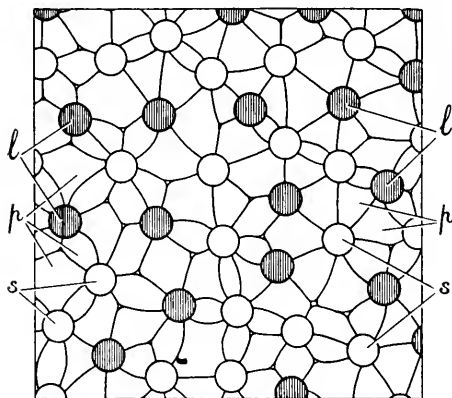


FIG. 28.—*Coprinus plicatilis*. Surface view of part of the hymenium which has shed its spores and is exhausted: *l*, the long basidia and the basidia of intermediate length, not here distinguished from one another but all shaded alike; *s*, the short basidia, left unshaded; *p*, the paraphyses, now expanded to the maximum extent. Material obtained at Kew, England. Magnification, 293.

and the gills of all other Coprini in that they are non-deliquescent. The free edges of the long gills are white with swollen sterile cells, and those of the short gills bear cheilocystidia (Fig. 24, p. 41).

The Hymenium.—The hymenium consists of paraphyses, basidia, and cystidia, and is typically coprinoid in structure.

The paraphyses are welded together laterally in the usual way so as to form a continuous membrane in which the basidia are inserted, and from five to eight paraphyses

surround each basidium and isolate it from its neighbours (Figs. 28 and 31).

The basidia are irregularly dimorphic-trimorphic: in some places on the hymenium they appear to be dimorphic, while in other places they are distinctly trimorphic (Figs. 29 and 31). The only other *Coprinus* in which I have observed basidial trimorphism is *C. niveus*. As a rule, each basidium bears four spores but, as in *C. niveus*¹ and other Coprini, one finds here and there, among the normal basidia, abnormal basidia bearing three or five spores (Fig. 30).

¹ These *Researches*, vol. ii, 1922, p. 320, Fig. 110, *b*, *c*.

The cystidia on the sides of the gills (pleurocystidia), in an unexpanded pileus, stretch nearly or completely across the inter-

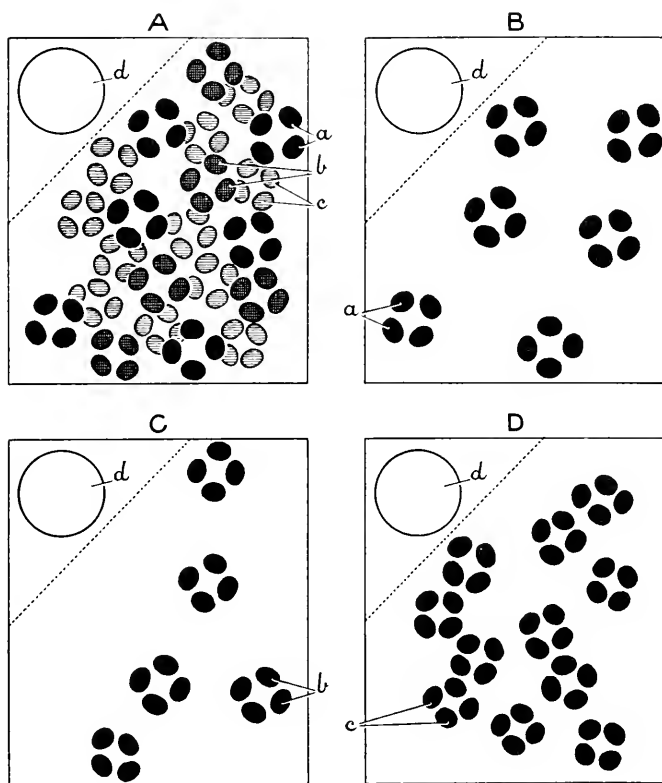


FIG. 29.—*Coprinus plicatilis*. A, a camera-lucida drawing showing the disposition of the spores and the appearance of a cystidium on the surface of a gill as seen in a surface view. The basidia are trimorphic: *a* (shaded black), the spores of the long basidia; *b* (cross-hatched), the spores of the basidia of medium length; and *c* (shaded with parallel lines), the spores of the short basidia. The spores *a* and *b*, in several places, stand over the spores *c*. The cystidium *d* was seen on another part of the hymenium. At B, C, and D, the spores of the long basidia, the intermediate basidia, and the short basidia respectively have been set out. Fruit-body obtained at Birmingham, England. Magnification, 426.

lamellar spaces; but their apices are not united to the paraphyses of the opposing gill, so that the pleurocystidia of *Coprinus plicatilis* differ from those of *C. atramentarius* and *C. lagopus* in that they do not lock adjacent gills together (Fig. 24, p. 41). However, they

doubtless function as guards and prevent adjacent gills from coming into contact during the development of the spores. Each cystidium (Fig. 31, *d*) has a thin cylindrical stalk about $20\ \mu$ long and a body which is ventricose below, becoming narrower and obtusely rounded above. The length of each isolated cystidium is $80\text{--}100\ \mu$ varying in very large fruit-bodies up to $150\ \mu$, while the breadth of the ventricose part of the body is $25\text{--}28\ \mu$. The pleurocystidia of *C. plicatilis*, relatively to those of *C. micaceus*, *C. lagopus*, and *C. niveus*, are but slender structures.

The ends of the short gills are free from basidia but bear cheilocystidia (Fig. 24, p. 41). These more or less resemble the pleuro-

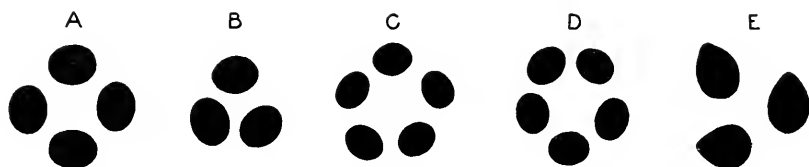


FIG. 30.—*Coprinus plicatilis*. Variations in number of spores on a basidium viewed from above. A, a normal basidium bearing four spores. B–D, abnormal basidia: B, with three spores; C and D, each with five spores. E, three isolated normal spores lying on one side in water. Fruit-body obtained at King's Heath, England. Magnification, 587.

cystidia in form but are smaller. The ends of the long gills bear swollen cells (Fig. 24, p. 41).

As the pileus expands, adjacent gills come to be moved widely apart; and, when expansion is just complete, the cystidia can be seen with the microscope projecting from the gill-sides as free pegs.

The Spores.—The spores are deep black, both *en masse* in a spore-deposit on white paper and when seen individually under the microscope. Their dimensions, relatively to the basidial axis, are three : length, breadth, and thickness. The average dimensions for fifty spores obtained from a single fruit-body which grew on a lawn at Birmingham, England, measured with the Poynting plate-micrometer, were $12.9 \times 10.7 \times 7.9\ \mu$.¹ When seen from in front or behind (*i.e.* when one looks from or toward the axis of the basidium), a spore appears heart-shaped or, in some fruit-bodies, spindle-shaped; and, when seen from above (*i.e.* when one looks

¹ These *Researches*, vol. i, 1909, p. 162.

down on the hymenium), it appears oval (Fig. 32, A and B). In different fruit-bodies the spores vary considerably in their size and to some extent in their shape (*cf.* A, B, and C in Fig. 32). While in most fruit-bodies the spores in front view are distinctly heart-

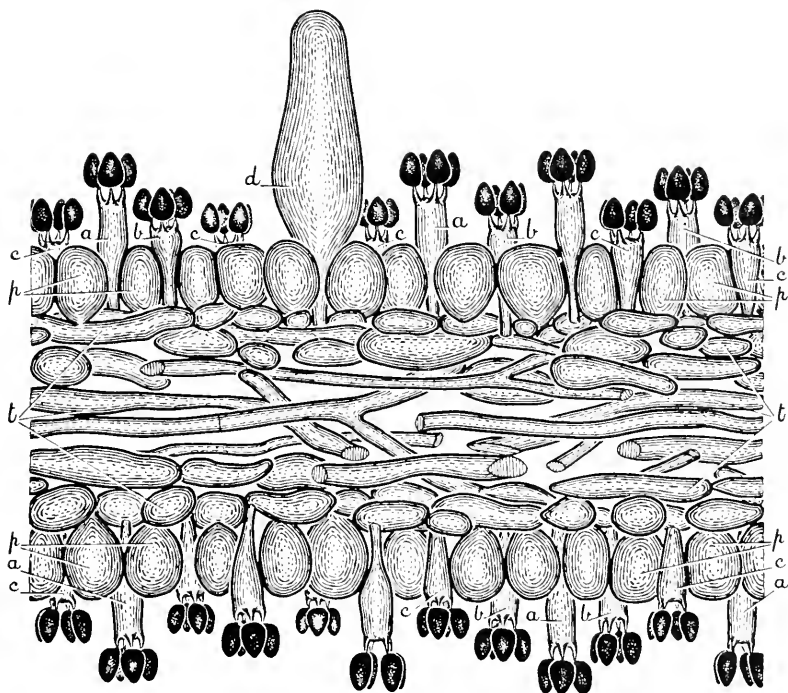


FIG. 31.—*Coprinus plicatilis*. Cross-section of part of a gill, to show the elements of which it is constructed. The hymenium consists of trimorphic basidia, sterile paraphyses, and cystidia: *a a*, long basidia; *b b*, basidia of medium length; *c c*, short basidia; *p p* paraphyses; *d*, a cystidium. The trama *t t* consists of a network of hyphae; there is no special well-marked-off subhymenium. Material obtained at King's Heath, England. Magnification, 426.

shaped, in some fruit-bodies they are spindle-shaped. In the extremely large fruit-body already mentioned in connection with a discussion of *Coprinus hemerobius*, the spores, although still somewhat broader than they were thick, were almost oval with the lower half only slightly wider than the upper half (Fig. 23, *d-f*, p. 39).

The rate of fall of the spores of *Coprinus plicatilis*, in still air, just after they have left the gills, was found for one fruit-body to

be 4.29 mm. per second.¹ In most Hymenomycetes, the rate of fall of the spores under similar conditions is much less than this. In *Psalliota campestris*, for example, it is only about 1.5 mm. per second.² The high rate of fall of the spores of *C. plicatilis* is doubtless due in part to their large size and in part to their high specific gravity which was determined to be approximately 1.21.³

The Stipe.—The stipe is very slender, fragile, smooth, hollow, greyish tinged with white or brown, and varying in height from 3 to 9 cm. Fruit-bodies with relatively short stipes were found on exposed well-lighted lawns and in well-cropped meadows, while

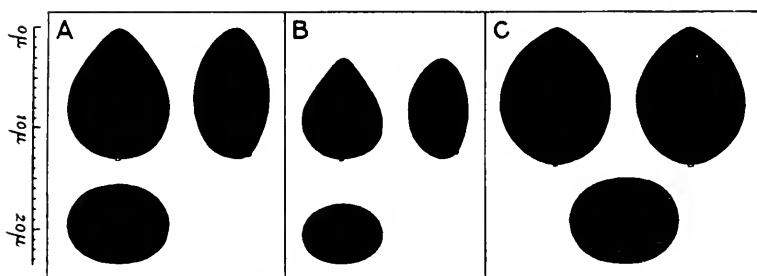


FIG. 32.—*Coprinus plicatilis*. Variations in size and shape of average spores of three different fruit-bodies taken from the same lawn at Birmingham, England : A in 1906, B in 1910, and C in 1911. A, front view, side view, and end view of an average spore (1906 fruit-body). B, similar views (1910 fruit-body). C, two front views and one end view of average spores (1911 fruit-body). Magnification, 1333.

fruit-bodies with relatively longer stipes were found under trees in damp and shady places. The base of each stipe is swollen. The shaft tapers upwards very slightly : it is usually 1.5–2 mm. thick above the swelling and about 1 mm. thick just below the pileus. Small pilei are borne on thinner stipes than large pilei. However, there is no uniform correlation between the width of an expanded pileus and the height of the stipe. Thus, the widths of the pilei of three fruit-bodies were 12 mm., 15.5 mm., and 39 mm., and the heights of the stipes 5.4 cm., 9 cm., and 5.5 cm. respectively.

Just before and during the expansion of the pileus, the apex of the stipe is very sensitive to the stimulus of gravity ; and, when a fruit-body with an upright stipe at this stage of development is

¹ These *Researches*, vol. i, 1909, p. 175. ² *Ibid.*, cf. p. 180. ³ *Ibid.*, p. 154.

removed from a lawn and set in a horizontal position, the end of the stipe soon turns the pileus upwards, thus bringing it once more into the optimum position for shedding its spores. During this reaction, one may observe geotropic swinging, just as in *Coprinus curtus*.¹

The Periodicity in Fruit-body Development and Time of Spore-discharge.—Whilst studying *Coprinus plicatilis* as it occurred on a lawn in my father's garden at King's Heath, England, I observed that, day after day during the summer and early autumn, new fruit-bodies made their appearance in a rhythmical manner. Early in the morning, after a dewy night, very young fruit-bodies could be seen springing up here and there on the turf. Soon the pilei began to expand, and spore-discharge took place at midday or very early in the afternoon. Late in the afternoon the fruit-bodies withered. Not once, although these observations were repeated during several successive years, did I ever have reason to suppose that pileus-expansion and spore-discharge took place at night.

The periodicity in fruit-body development and spore-discharge in *Coprinus plicatilis* resembles that of *C. curtus*, described in the last Chapter. It is doubtless due to the diurnal changes of light and darkness; and it may well be that in *C. plicatilis*, just as in *C. curtus*, the final development of each fruit-body is dependent on a morphogenic stimulus given by light to the rudiment of the fruit-body during one or more days preceding the day the pileus expands and liberates its spores.

The Ripening and Discharge of the Spores and the Absence of Autodigestion.—During their development, the gills of *Coprinus plicatilis*, like those of *C. comatus*, *C. atramentarius*, and all other Coprini, darken in colour from below upwards (Fig. 33). This field observation and other observations made with the microscope on freshly-gathered developing fruit-bodies have convinced me that in *C. plicatilis* the spores on each gill ripen from below upwards. By the time that pilear expansion begins, the gills are uniformly black, owing to all the spores having become fully pigmented. As soon as the expansion of the pileus (Fig. 25, p. 42) has been completed, spore-discharge begins.

¹ Vide these *Researches*, vol. i, 1909, pp. 65–67, Fig. 24.

The discharge of the spores of *Coprinus plicatilis* takes place, as in *C. comatus*, *C. atramentarius*, and all other Coprini, on each gill from below upwards (Fig. 34). This fact can be made out both macroscopically and microscopically. In the field one can observe that, during the spore-discharge period, the gills become lighter from below upwards, or, from the point of view of the pileus as a whole, centripetally; and, if one examines a whitening gill with the microscope, one finds that all the basidia in its upper darker part still bear spores on their sterigmata, that all the basidia in its

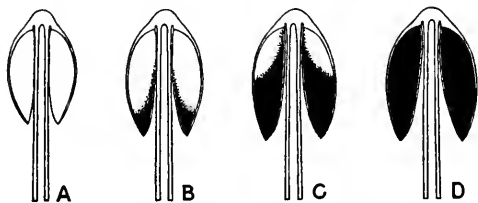


FIG. 33.—*Coprinus plicatilis*. Vertical sections showing four stages in the development of a young and unexpanded fruit-body, drawn semi-diagrammatically, to illustrate the ripening of the spores from below upwards on the gills. A, spores on gills developing but as yet colourless, so that gills are white. B and C, spores on gills ripening from below upwards and thus turning the gills black from below upwards. D, the spores have now all developed their pigment, so that the gills have become completely blackened. Enlarged to 1.33 the natural size.

lower lighter part have shed their spores, and that between the darker and the lighter parts there is an intermediate zone of spore-discharge where the basidia are engaged in shooting away their spores. This zone of spore-discharge, which travels slowly toward the disc of the pileus, in general resembles that of other Coprini, e.g. *C. comatus* and *C. atramentarius*, but it is somewhat broader

than usual. Doubtless, although the point has not yet been verified by actual observation, where the basidia are trimorphic the zone of spore-discharge consists of three sub-zones: an upper, in which only the longest basidia are discharging their spores; an intermediate, in which only the basidia of intermediate size are discharging their spores; and a lower, in which only the shortest basidia are discharging their spores. In most Coprini, e.g. *C. comatus*, *C. sterquilinus*, *C. atramentarius*, *C. lagopus*, and *C. curtus*, the basidia are strictly dimorphic and the zone of spore-discharge consists of two sub-zones only; but in *C. micaceus* the basidia are quadrimorphic, and the zone of spore-discharge consists of as many as four sub-zones. In having trimorphic basidia and three sub-zones in the zone of spore-discharge, *C. plicatilis* and *C. niveus*

occupy an intermediate position between *C. micaceus* and the other Coprini.

Although in *Coprinus plicatilis* the spores ripen and are discharged on each gill from below upwards, during the spore-discharge period the gills—as already remarked by systematists—remain “dry” and “non-deliquescent,” i.e. those parts of the gills which have shed their spores are not gradually destroyed from below upwards by autodigestion (Fig. 34, A–E). This curious absence of autodigestion, which places *C. plicatilis* in a unique position among the Coprini, I have verified again and again by examining living fruit-bodies both with the naked eye in the field and with the microscope in the laboratory. Macroscopically, it is quite easy to observe that the gills of a fruit-body are present in their entirety during the whole of the spore-discharge period and when this has just come to an end (Fig. 34, E).

It is possible to observe the progress of spore-discharge in any particular fruit-body in the field by merely looking down on the pileus when this has just completed its expansion. Immediately before spore-discharge begins, the pileus, owing to the presence of the black spores on all the basidia on the cleft gills, is uniformly grey. As soon as spore-discharge begins, a white zone

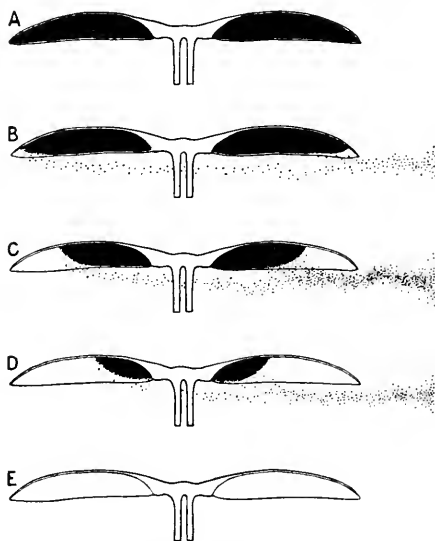


FIG. 34.—*Coprinus plicatilis*. Vertical sections of a pileus, showing a series of successive stages, drawn semi-diagrammatically, to illustrate the fact that in this species the discharge of the spores is not accompanied by any autodigestion of the gills. A: the pileus has just become completely expanded and the gills are about to begin to discharge their spores. B: half an hour later; spore-discharge is taking place from below upwards on each gill and centripetally in respect to the pileus as a whole. C: about an hour later than B; spore-discharge is at its maximum activity. D: about an hour later than C; spore-discharge nearing completion but still very active. E: about an hour later than D; spore-discharge has been completed. As shown in B–E, during spore-discharge, the gills have remained dry and undigested. Enlarged to 1.33 the natural size.

appears at the pileus-periphery and, as spore-discharge continues, the white zone increases in width centripetally, thus gradually diminishing the central grey zone. Finally, when all the spores have been shed, the white zone extends to the disc and the grey zone has disappeared. If one gathers the fruit-body at the end of the spore-discharge period, one finds the gills whitened owing to the loss of their spores but completely intact, owing to the fact that they have not been subjected to the process of autodigestion (Fig. 34, E).

At Kew Gardens on a wet lawn, early in September, 1925, at midday, there were many fruit-bodies with expanded pilei among the grass; and, by looking down on them and observing their white and grey zones, I was able to assure myself that, at one and the same moment, in some of the fruit-bodies the process of spore-discharge had not yet begun, in others it was from one-quarter to one-half completed, and in yet others it was nearly complete. Then I gathered the fruit-bodies and examined their pilei from below. The white and grey zones seen directly on each gill were found to correspond exactly with the white and grey zones which had been seen by looking down on the pilei from above.

The spores of *Coprinus plicatilis*, in England, in the month of September, are shed from shortly before noon to late in the afternoon. The spore-discharge period is a brief one and, other things being equal, is determined by the size of the pileus. The longer the gills, the longer the distance the zone of spore-discharge has to travel before all the spores are discharged and the longer the time occupied by the journey. Therefore here, as in *Coprinus curtus* and indeed all other Coprini, the larger the pileus, the longer is the spore-discharge period, and the smaller the pileus, the shorter is the spore-discharge period. The actual length of the spore-discharge period, which has not yet been determined by precise observations on particular fruit-bodies, was estimated to be from about one to four hours according to the size of the pilei concerned.

The four spores of each basidium of *Coprinus plicatilis* are discharged from their sterigmata in succession in the course of a few seconds or minutes, and each discharge is accompanied by drop-excretion at the spore-hilum, just as in all other Hymenomycetes. I gathered some fruit-bodies from a lawn at Kew Gardens, took them to the Herbarium, removed the pilei, turned the pilei upside

down on a slide, and observed the discharge of the spores with the low power of the microscope. Mr. W. B. Grove examined one of the pilei in the same manner and kindly observed the times of discharge of all the four spores of each of five basidia. If the time of discharge of the first spore of each basidium be taken as the zero of the time-scale and the times of discharge of the other three spores be reckoned from it, then the accompanying Table shows the times of discharge for all the four spores of each of the five basidia.

The Rate of Discharge of the Four Spores for each of Five Basidia of Coprinus plicatilis

Basidium	Times in Seconds at which the Four Spores were Discharged			
	First Spore	Second Spore	Third Spore	Fourth Spore
No. 1	0	3	5	6
No. 2	0	3	5	15
No. 3	0	5	7	8
No. 4	0	7	9	10
No. 5	0	5	11	14
Average for the five basidia	0	4·6	7·4	10·6

From the Table it will be seen that, when once a basidium had begun to shoot away its spores, on the average the last of its spores was discharged within 11 seconds of the first. In *Coprinus sterquilinus*, by observations made on five basidia, it was determined that on the average the last of the four spores was discharged within 68 seconds of the first.¹ The interval of time observed between first and last discharges in *C. plicatilis*, therefore, was only one-sixth of that observed in *C. sterquilinus*.

Coprinus plicatilis was one of the first Hymenomycetes which I examined when attempting to determine whether or not spores are violently discharged from their basidia. A gill was placed flat in a closed compressor cell, so that the hymenium looked upwards. A plane in the air about 0·1 mm. above the plane in which the spores were disposed was focussed with the microscope. When the discharge of the spores was taking place, many spores came momentarily

¹ These *Researches*, vol. iii, 1924, p. 245.

into view. Clear proof was thus obtained that the basidia were shooting their spores more or less perpendicularly upwards.¹

A few seconds before the discharge of a spore a drop is excreted at the spore-hilum, just as in all other Hymenomycetes. Occasionally, after a spore and its drop have been discharged together, a new drop is excreted at the end of the vacant sterigma. This kind of abnormality I have observed also in *Coprinus Rostrupianus* and *C. curtus* (Fig. 20, E and F, p. 31). Excessive drop-excretion from the spore-hila of the four spores of a single basidium, followed by coalescence of the drops into one large drop and non-discharge of the spores, takes place under very moist conditions, as in *Panaeolus campanulatus*² and *Coprinus sterquilinus*.³

As we have seen, just before a pileus of *Coprinus plicatilis* expands, there are a number of cystidia projecting across the interlamellar spaces and from the margins of the shorter gills. In *C. atramentarius*, *C. lagopus*, etc., each pleurocystidium suffers autodigestion shortly before the upward-travelling zone of spore-discharge arrives at its level. In *C. plicatilis*, although during the spore-discharge period the gills as wholes are not destroyed from below upwards by autodigestion, we may yet ask whether or not the cystidia are destroyed by autodigestion in succession from below upwards as in the species just named. In an endeavour to answer this question I gathered some large fruit-bodies which were shedding spores and immediately examined the under sides of the pilei with the low power of the microscope. Apparently the cystidia along the gill-margins and on the gill-sides were disappearing in fruit-bodies beginning to discharge their spores, and had already disappeared in fruit-bodies in which spore-discharge was coming to a close; and two pleurocystidia were seen to excrete a drop of fluid and then to retire to the gill-side. These observations suggest that the pleurocystidia of *C. plicatilis* are actually autodigested in the same manner as those of *C. atramentarius*⁴ and *C. lagopus*; but, before this conclusion can be accepted without reservation, it

¹ These *Researches*, vol. i, 1909, p. 142.

² *Ibid.*, vol. ii, 1922, p. 308, Fig. 104.

³ *Ibid.*, vol. iii, 1924, pp. 247-250, Fig. 106.

⁴ For illustrations of autodigesting cystidia of *C. atramentarius*, vide these *Researches*, vol. iii, 1924, p. 289, Fig. 123.

is desirable that the autodigestion of a number of individual pleurocystidia should be followed in detail with the microscope.

From a pileus which has begun to shed its spores one may obtain a black spore-deposit on white paper within a few minutes. Under field conditions, the spore-clouds are carried away from beneath the pilei by the wind. As already pointed out, owing to the fact that the spores of *Coprinus plicatilis* are relatively large and have a high specific gravity, in still air they fall more rapidly than those of most other Hymenomycetes.¹ In a gentle breeze, before they settle, they are therefore not likely to be carried to so great a distance as the spores of *Psalliota campestris*, *Marasmius oreades*, and many other grassland agarics.

The Generic Position of *Coprinus plicatilis*.—In a special paper,² published in 1919, and in Volume III of these *Researches*,³ in the course of some critical remarks on the generic position of certain Agaricineae, it was shown that, although *Coprinus plicatilis* has lost one of its chief *Coprinus* characters, namely, autodigestion of the gills, all its other characters are typically coprinoid; and it was concluded, contrary to the view of Masee, that the fungus must be retained in the genus *Coprinus*. This conclusion has been supported and strengthened by the detailed account of the structure and mode of functioning of the fruit-body of *C. plicatilis* which has just been given.

The Absence of Autodigestion and its Significance.—In the *Coprini* in general, in respect to the liberation of the spores, the autodigestion of the gills is a mechanically advantageous process: on each gill it proceeds from below upwards and destroys those parts of the gills which have already shed their spores and which, if they continued in existence, would hinder the fall of the remaining spores.

It is probable that the ancestors of *Coprinus plicatilis* had gills which underwent autodigestion in the manner which is typical for the genus *Coprinus*, but that this character was lost by *C.*

¹ *Vide supra*, p. 48.

² A. H. R. Buller, "Some Critical Remarks on the Generic Positions of *Psathyra urticaecola* Berk. et Broome, *Coprinus plicatilis* Fr., and *Psathyrella disseminata* Pers.," *Trans. Brit. Myc. Soc.*, for 1916, vol. v, 1917, pp. 482-489.

³ These *Researches*, vol. iii, 1924, pp. 137-140.

plicatilis either at the time its fruit-body took on its present peculiar form or subsequently thereto. In inaequi-hymeniiferous fruit-bodies which before spore-discharge begins do not open like a parasol, especially those with large gills, *e.g.* those of *C. comatus*, *C. atramentarius*, *C. picaceus*, *C. niveus*, and *C. stercorarius*, the loss of autodigestion would be disastrous to the functional efficiency of the pilei, for the undigested portion of the gills would certainly prevent the great majority of the spores from ever escaping into the outer air. On the other hand, in *C. plicatilis*, owing to the parasol-like opening of the pileus before spore-discharge begins, the small depth of the gills, and the splitting of the gills from above downwards so that in expanded pilei they are Y-shaped in cross-section, the loss of autodigestion in respect to the liberation of the spores is of much less importance. The hymenium on the upper split portion of each gill, like that on the solid wedge-shaped gills of the Aequi-hymeniferae, everywhere looks downwards and, on this account, all the spores which it bears can be liberated without any mechanical hindrance whatever. The unsplit parallel-sided lower portion of each gill, although ageotropic and therefore not in possession of any means for placing itself in a vertical plane, is nevertheless situated almost in a vertical plane, owing to the negatively geotropic reaction of the stipe which sets the whole pileus in a horizontal plane and therefore all the gills in almost vertical planes. In an expanded pileus the interlamellar spaces are very broad and the unsplit portions of the gills are not much more than 1 mm. high. If the lower half of each gill in an expanded pileus is not quite vertical, so that one side looks slightly upwards and the other side slightly downwards, the side looking downwards can discharge its spores so that they all escape from the fruit-body. The side looking upwards is at a disadvantage: if it looks upwards only very slightly, some or possibly nearly all its spores will be able to escape from the fruit-body; but, if it looks upwards in a greater degree, only a few spores near the margin of the gill will escape and the rest of the spores, after being shot into the air, will fall back on to the gill and stick there. In the field, in the afternoon, one often finds exhausted fruit-bodies with some of the gills powdered black on one of their two sides, and this doubtless is due to the want of

verticality of the gills in question. If such powdered gills during the spore-discharge period had been subjected to the process of autodigestion as in other Coprini, the spores which fell on to their upper sides might have escaped freely from the fruit-body. We



FIG. 35.—*Coprinus longipes*. Three young fruit-bodies coming up spontaneously on old horse-dung in a laboratory culture at Winnipeg. One of them is expanding its pileus. The unexpanded pilei were brown. Natural size.



FIG. 36.—*Coprinus longipes*. Three fruit-bodies with rather short stipes and fully expanded pilei on horse dung coming up spontaneously in a laboratory culture at Winnipeg. The pilei, like those of *C. plicatilis*, are broadly convex, radially plicate, and depressed at the disc. Natural size.

thus perceive that in *C. plicatilis* the absence of autodigestion is a disadvantage to the working of the fruit-body mechanism, but not a very serious one; for, even with this handicap, most of the spores escape from the pileus and are carried off by the wind. The pileus of *C. curtus*, in opening out like a parasol prior to the beginning of spore-discharge, resembles that of *C. plicatilis* and, if its gills ceased to undergo autodigestion during the spore-discharge period, it is not improbable that, like the pileus of *C. plicatilis*, it would

still be able to liberate a sufficient number of spores to maintain the species in existence.

Under moist conditions, the expanded pileus of *Coprinus plicatilis* remains convex, whereas that of *C. curtus* becomes plane and then more or less revolute. This is correlated with the fact that in the former species the lower halves of the gills are not destroyed by autodigestion from below upwards, whereas in the latter species

they are. In *C. plicatilis*, since the gills remain intact, the revolution of the pileus is rendered mechanically impossible.

Coprinus plicatilis and Coprinus longipes.—In 1929 I described in detail a new species of *Coprinus* which had come up many times on horse dung kept for some weeks or months in culture dishes in the laboratory at Winnipeg, and I named it *Coprinus longipes* because its stipe is often very long in proportion to the width of the pileus.¹ This species is illustrated for the first time in Figs. 35–39. Fig. 35 shows three



FIG. 37.—*Coprinus longipes*. A single fruit-body coming up spontaneously on an old horse-dung ball in a laboratory culture at Winnipeg. The pileus has been turned so as to exhibit the radial plications and dark central depressed disc. Natural size.

young fruit-bodies on a horse-dung ball, one of which is expanding its pileus and becoming plicate. Fig. 36 shows three fruit-bodies with rather short stipes and pilei which are fully expanded. The broadly convex pilei with their darker depressed discs and radial plications greatly resemble those of *C. plicatilis* (cf. Figs. 21 and 22, pp. 35 and 37). Fig. 37 shows a single fruit-body on the side of a horse-dung ball with the pileus turned so as to exhibit the dark central depressed disc. Fig. 38 shows a group of four mature fruit-bodies and an isolated pileus, all removed from a large culture dish which contained horse dung.

¹ G. R. Bisby, A. H. R. Buller, and J. Dearness, *The Fungi of Manitoba*, London, 1929, p. 118.

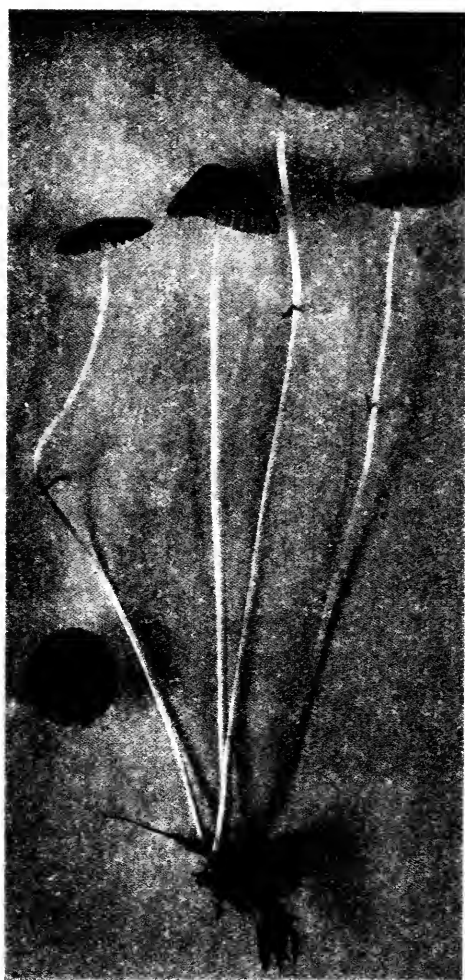


FIG. 38.—*Coprinus longipes*. A group of four fruit-bodies which came up spontaneously, removed from old horse dung contained in a laboratory culture dish at Winnipeg. The pilei are fully expanded and the stipes very long. An isolated pileus seen from above, on the left. Natural size.



FIG. 39.

FIG. 39.—*Coprinus longipes*. The largest fruit-body observed. Its stipe is 15 cm. long and its pileus 2.25 cm. in diameter. Removed from old horse dung on which it came up spontaneously in a culture dish at Winnipeg. Natural size.

Here the stipes are very long. Finally, Fig. 39 shows the largest fruit-body that has been observed. Its stipe is 15 cm. long and its pileus 2.25 cm. broad.

Coprinus longipes, as I remarked,¹ "resembles *C. plicatilis* in general appearance and might be mistaken for it; but it differs in coming up on horse dung instead of in grassy places, in having a slightly smaller depressed disc, in having gills which waste or deliquesce at their edges instead of remaining entire, and in having a stipe which is usually longer."

Coprinus longipes is of interest to us here owing to the fact that, while in its general appearance and many of its histological details it greatly resembles *C. plicatilis*, the edges of its gills but not the whole of its gills undergo a distinct although a very limited amount of autodigestion. Thus *C. longipes* serves to connect *C. plicatilis*, the gills of which do not undergo autodigestion, with the typical Coprini, e.g. *C. comatus*, *C. niveus*, and *C. stercorearius*, in which the autodigestion of the gills is a very marked characteristic.

A Taxonomic Description of *Coprinus plicatilis*.—The description of *Coprinus plicatilis* which follows is based on my own studies of this species, and has been drawn up with a view to its being of some use to taxonomists. The taxonomic descriptions of *C. plicatilis* hitherto given have all been relatively brief and incomplete. Massee,² in his *British Fungus-Flora*, describes the pileus as "eventually becoming plane, the margin splitting and revolute;" but this is erroneous, for the pileus when fully expanded is broadly convex while its margin usually remains unsplit and is never revolute. The same author,³ in *A Revision of the Genus Coprinus*, does not mention the fact that cystidia are present on the sides of the gills of *C. plicatilis*, and he fails to show any cystidia in his drawing of a transverse section of the pileus in which whole gills and the details of their hymenium are represented. Perhaps the most important omission from previous descriptions of *C. plicatilis* is that the gills remain dry and are not destroyed by deliquescence.

¹ G. R. Bisby, A. H. R. Buller, and J. Dearness, *The Fungi of Manitoba*, London, 1929, p. 118.

² G. Massee, *British Fungus-Flora*, London, 1892, vol. i, p. 328.

³ G. Massee, "A Revision of the Genus *Coprinus*," *Annals of Botany*, vol. x, 1896, p. 177, Plate X, Fig. 25.

COPRINUS PLICATILIS *Fr.*

Pileus before expanding 6–10 mm. high, 5–8 mm. broad, ovate-cylindric, striate to the disc, without scales and usually without hairs, brown, often darker at the apex, on expanding becoming broadly convex but never becoming quite plane or revolute, the margin directed obliquely downwards until withering; when fully expanded 12–22 mm. broad, varying up to 30 or even 40 mm.; the disc 4–6 mm. broad, varying up to 10 mm., brown, usually darker than the rest of the pileus, distinctly depressed; the convex sides of the pileus greyish-brown and beautifully plicate owing to the splitting of the gills from above downwards, without any hairs or scales. Stipe 3·5–9·0 cm. long, often 4–6 cm., swollen at the extreme base, above evenly cylindric, 1·0–2·0 mm. thick, dull white or white tinged with brown, smooth, hollow, at the point of attachment in the expanded fruit-body separated from the gills by a bare zone of pileus-flesh 0·5–2·0 mm. wide. Gills distant from one another, never locked together by bridging cystidia, black, at the end of the spore-discharge period often powdered black on one side, in the fully expanded pileus horizontally extended, narrow, broadly arched above where attached to the flesh, almost straight below, usually 6–10 mm. long but varying in the largest fruit-bodies up to 17 mm. long, 1·5–3·0 mm. broad, the inner ends united to form a collar separated from the stipe by the zone of bare flesh already mentioned, not undergoing autodigestion from below upwards and thus persisting dry and intact during the spore-discharge period and until the whole fruit-body collapses. Flesh thick at the disc, over the gills very thin, at first furrowed but soon split into rays as the pileus opens and the gills become cleft from above downwards, date-brown, lighter as drying takes place. During the ripening of the spores, the gills become progressively black from below upwards. During the discharge of the spores, the gills become progressively dull white from below upwards and toward the stipe; so that, in the field, when one looks down on the wheel-like translucent pileus shedding spores, one can detect how far spore-discharge has advanced by noting the breadth of the outer white zone as compared with the diameter of the central

black zone. Spore-discharge period 1–4 hours, varying with the size of the fruit-body, in progress in the late morning or early afternoon, never at night. Spores black in the mass and jet-black under the microscope, smooth, rounded heart-shaped to spindle-shaped, pointed at the apex, with three differing dimensions, $12.9 \times 10.7 \times 7.9 \mu$ (average of 50 spores on one fruit-body), but in some fruit-bodies distinctly smaller (10μ long) or even larger (13.2μ long), rather variable in size and shape. Basidia of two or three differing lengths, each one surrounded by 5–8 paraphyses. Cystidia on the sides of the gills with a thin cylindrical stalk between the paraphyses, ovoid-tapering, obtusely rounded at the free end which is never united with the paraphyses of the opposing gill, $80\text{--}100 \mu$ long, varying up to 150μ , $25\text{--}28 \mu$ broad, disappearing during the spore-discharge period. Common in England, Europe, United States of America, Canada, etc., in damp, grassy places in woods, in close-cropped pastures, on mown lawns, on grass borders along roadways, etc., not recorded on dung; during damp weather in summer and autumn a new crop comes up each morning, sheds spores about midday, and withers by the evening.

This species is characterised by coming up in grassy places, by its expanded, wheel-like, translucent pileus remaining broadly convex and never becoming quite plane or revolute, by the inner ends of the gills being united to form a slight collar separated from the stipe by a bare zone of flesh, by the deep-black more or less compressed heart-shaped spores, and particularly by the gills never undergoing autodigestion (deliquescence) but remaining dry and intact during spore-discharge and until the whole fruit-body withers.

Except for the absence of autodigestion, the gills are typically those of a *Coprinus*; for, as in the gills of all other *Coprini*, the paraphyses are strongly developed, the basidia are heteromorphic as regards length and unequal in protuberancy, and the spores ripen on the gills and become discharged from the gills in succession from below upwards. The nearest relative of *C. plicatilis* appears to be *C. longipes* Buller, a species observed in central Canada, which differs in occurring on horse dung and in having gills which at their margins show a slight wasting or autodigestion during the spore-discharge period.

CHAPTER III

VARIOUS REMARKS ON THE COPRINI

Comparative Remarks on the Coprinus and Non-Coprinus Types—Coprinus Ink—
The Hyphal String in the Stipe of *Coprinus comatus*—A Further Remark on
the Pileus-flesh of *Coprinus comatus*—A Giant Fruit-body of *Coprinus comatus*
—Excretion of Drops of Water from a Pileus of *Coprinus niveus*—*Coprinus*
lagopus growing on Beet Seeds

Comparative Remarks on the Coprinus and Non-Coprinus Types.—
The chief points in the mechanism for the production and liberation of spores in the Inaequi-hymeniiferous and Aequi-hymeniiferous Types were set forth in Volumes II and III.¹ Now that all the Sub-types have been described it will be convenient to compare the Types from the point of view of their efficiency in the production and liberation of the spores.

Since the Psathyrella and Bolbitius Sub-types contain but very few species and evidently stand near to certain of the Coprinus Sub-types, in what follows species of the large, important, and more contrasting Panaeolus and Armillaria Sub-types will be chosen to represent the Aequi-hymeniiferous Type.

There can be no doubt that, in species with spores of about equal size, the number of spores produced per unit area of the hymenium is greater in the Non-Coprinus Type than in the Coprinus Type. This point is illustrated graphically in Fig. 40. In this Figure, A shows a piece of the exhausted hymenium of *Stropharia semiglobata* and B an equal area of the exhausted hymenium of *Coprinus comatus*, while C and D show the total number of spores (one for each sterigmatic stump) which were produced by A and B respectively. A count of the spores enables us to conclude that : the number of spores of A : the number of spores of B : : 188 : 68,

¹ These *Researches*, vol. ii, 1922, pp. 239–241 ; vol. iii, 1924, pp. 118–120.

i.e. on a unit area of the hymenium *Stropharia semiglobata* produces nearly three times as many spores as *Coprinus comatus*. In other words, in the example chosen, as judged by the criterion of

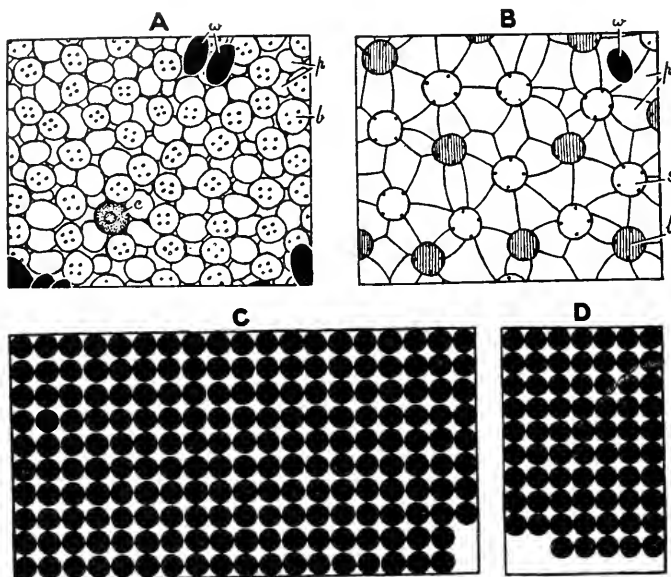


FIG. 40.—Comparison of the number of spores produced on equal areas of hymenium in the *Panaeolus* and *Comatus* Sub-types of hymenial organisation. A, exhausted hymenium of *Stropharia semiglobata*. The basidia, *b*, are all collapsed and are known by their sterigmatic stumps; *p*, paraphyses; *c*, a cystidium; *w*, wasted spores. B, exhausted hymenium of *Coprinus comatus*. The long basidia, *l*, and the short basidia, *s*, each have four sterigmatic stumps; *p*, paraphyses; *w*, a wasted spore. C shows a mass of spores equal in number to the sterigmatic stumps in A; while D shows a mass of spores equal in number to the sterigmatic stumps in B. It is obvious from an inspection of C and D that the *Stropharia* produced about three times as many spores as the *Coprinus* per unit area of the hymenium. Magnification, 352.

the number of spores produced per unit area of the hymenium, the Non-Coprinus Type is about three times as efficient as the Coprinus Type. The difference in the number of spores produced per unit area in the two Types is fundamental and is correlated with the fact that on any small area of the hymenium, *e.g.* 0.1 square mm., in the Non-Coprinus Type several generations of basidia develop and liberate their spores in succession during a period of several

days, whereas in the *Coprinus* Type all the spores that are to be discharged must be ripe at approximately one and the same time, as they are all ultimately discharged and then the hymenium beneath them is destroyed by autodigestion in the course of a very few minutes.

In cross-section, the gills of the Non-*Coprinus* Type are wedge-shaped, whereas those of the *Coprinus* Type are parallel-sided or subparallel-sided. This fundamental difference is correlated with the fact that, in fruit-bodies with gills of equal depth, the gills of the Non-*Coprinus* Type are considerably more massive than those of the *Coprinus* Type. This is illustrated in Fig. 41, where A, B, and C are cross-sections of the gills of *Coprinus atramentarius*, *Collybia platyphylla*, and *Psalliota campestris* respectively, all drawn to the same scale. The gills of the *Coprinus* and of the *Collybia* are of the same depth, namely, 12 mm., and inspection shows at once that a single gill of the *Collybia* is equal in mass to several gills of the *Coprinus*.¹ In other words, with equal masses of material employed in the production of gills, the fruit-body of the *Coprinus* Type produces more gills than that of the Non-*Coprinus* Type. Hence we may conclude that per unit mass of the gills the area of hymenium supported is much greater in the *Coprinus* Type than in the Non-*Coprinus* Type. Therefore, if the number of units of area of the hymenium produced per unit of mass of the gills be taken by itself as the criterion for efficiency in fruit-body construction, the *Coprinus* Type may be said to be several times as efficient as the Non-*Coprinus* Type.

From the preceding discussion it is obvious that the advantage of the Non-*Coprinus* Type over the *Coprinus* Type in the number of spores produced per unit area of the hymenium is counterbalanced by the disadvantage that the gills of the Non-*Coprinus* Type must of necessity be much more massive than those of the *Coprinus* Type.

The pileus-flesh of the Non-*Coprinus* Type is, as a rule, much

¹ A paper cross-section of a gill of the *Coprinus* and another of a gill of the *Collybia*, each twelve times the natural size, were weighed separately against paper squares (each 5 mm. square), and it was found that the cross-sectional area of a gill of the *Collybia* was approximately seven times as great as the cross-sectional area of a gill of the *Coprinus*.

thicker than that of the *Coprinus* Type. This difference is at once apparent when one compares a median vertical section of a fruit-body of *Coprinus atramentarius* (Vol. III, Fig. 118, p. 278) or of

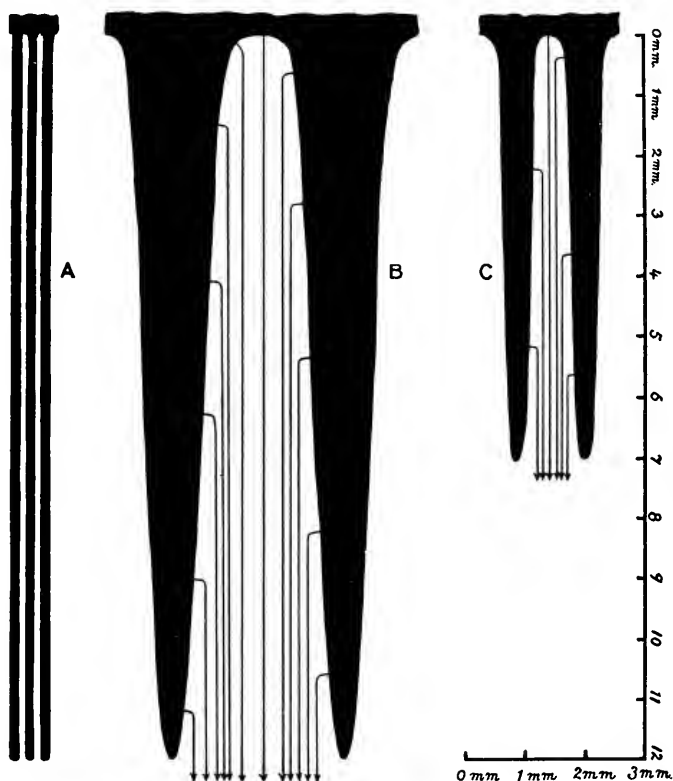


FIG. 41.—Relative shape and thickness of gills seen in cross-section, shown somewhat diagrammatically. A, *Coprinus atramentarius*, as an example of the *Coprinus* Type (the bridging cystidia omitted). B, *Collybia platyphylla* and C, *Psalliota campestris*, as examples of the Non-*Coprinus* Type. The arrows in B and C show the trajectories of a few spores discharged from the hymenium in still air. Magnification, 8.

C. comatus (Fig. 46 ; also Vol. I, Plate I, Fig. 1) with a similar section of *Psalliota campestris* (Vol. I, Plate I, Fig. 2) or of *Marasmius oreades*.

A *Psalliota campestris* fruit-body 8 cm. in diameter was compared with a *Coprinus comatus* fruit-body 12 cm. high. It was found that : (1) the hymenial area on the gill surfaces was about

195 square inches in the Mushroom and about 616 square inches in the *Coprinus*; and that (2) the pileus-flesh of the Mushroom was about eight times the volume of that of the *Coprinus*.

Let A = area of hymenium and V = volume of pileus-flesh. Then

A of the Mushroom : A of the *Coprinus* :: 1 : 3,

and V of the Mushroom : V of the *Coprinus* :: 8 : 1.

Therefore $\frac{A}{V}$, the ratio of the hymenial area to the volume of the pileus-flesh, is 24 times greater in the *Coprinus* than in the Mushroom. In other words, for every unit of volume of pileus-flesh the *Coprinus* produces 24 times the area of hymenial surface produced by the Mushroom, or for every unit of area of hymenial surface the Mushroom develops 24 times the volume of pileus-flesh developed by the *Coprinus*. This well illustrates the extraordinary economy in pileus-flesh effected in a large fruit-body of the *Coprinus* Type as compared with a large fruit-body of the Non-*Coprinus* Type. The small amount of flesh in large *Coprinus* fruit-bodies is a fundamental characteristic and is correlated with the fact that the gills become lighter in weight as the pileus opens out owing to their destruction by autodigestion.¹

The advantage which a large *Coprinus comatus* fruit-body has over a large *Psalliota campestris* fruit-body in its ratio of hymenial area to volume of pileus-flesh is to a large extent counterbalanced by the disadvantage that, per unit of area of the hymenium, the *Coprinus* produces a much smaller number of spores than the Mushroom. In other words: in the Mushroom, while a relatively large amount of pileus-flesh is developed for each unit of area of hymenium, each unit of area of the hymenium (owing to its producing many successive generations of basidia and owing to the small size of its hymenial elements and spores) develops a relatively very large number of spores, *i.e.* several times as many as there are on each unit of area in the *Coprinus*. If, therefore, the number of spores produced per unit volume of fruit-body material be taken as a criterion of fruit-body efficiency, then a large *Coprinus comatus* fruit-body and a large *Psalliota campestris* fruit-body may well be

¹ Cf. these *Researches*, vol. i, 1909, p. 214.

almost equally efficient, with the advantage, if any, lying with the *Coprinus*.

The fruit-body in the Agaricineae is, as already often stated, an organ whose one great function is the production and liberation of spores. The *Coprinus* Type and the Non-*Coprinus* Type of fruit-body resemble two variations on a single musical theme. Each performs its function in its own way; and so successful are both modes of operation that, under natural conditions, *Coprinini* and Non-*Coprinini* often exist side by side on the same substratum, and there is no reason to



FIG. 42.—*Coprinus sterquilinus*. A fruit-body in a very late stage of development, growing on sterilised horse dung in a glass dish. The pileus has become torn into rags, some of which are weighed down by dark drops—the liquid products of the autodigestion of the gills. Most of the spores have been shot away into the air, and a dark spore-deposit can be seen on the lower part of the stipe and on the top of the dung. Much of the liquid products of autodigestion has evaporated from the gill-edges. When a fruit-body, about to expand and shed its spores, is cut down and placed in an unnatural position in a small closed chamber, the liquid products of autodigestion and the black spores become mixed, thus forming an inky fluid. Reduced to two-thirds the natural size.

suppose that, at the present day, one Type is gaining at the expense of the other.

Coprinus Ink.—Owing to the autodigestion of the gills, large fruit-bodies of *Coprinus atramentarius*, *C. comatus*, *C. picaceus*, *C. sterquilinus*, etc., when kept in a closed vessel, give rise to a considerable quantity of a black and inky-looking fluid. Hence the larger Coprini are popularly known in English-speaking countries as *Ink Fungi* and in France as *Encriers* or *Bouteilles à l'encre*.

Written
with
Coprinus Ink.

FIG. 43.—The words were written with a brush dipped in the juice of an autodigested pileus of *Coprinus sterquilinus*. In the main, the blackness of the ink is due to the presence of the innumerable black spores. Reduced to two-thirds the original size.

Boudier named the black fluid *encre de Coprin*, and we may refer to it as *Coprinus ink*.

The dark colour of *Coprinus* ink is due chiefly to the innumerable black spores which it contains, but also to some extent to the dark colour of the fluid in which the spores are suspended. A fruit-body of *Coprinus sterquilinus*, which came up on horse dung in the laboratory (cf. Fig. 42), was removed from its substratum and was allowed to undergo autodigestion in a closed glass vessel. The *Coprinus* ink which was developed contained vast numbers of very black spores and was used to write the words shown in Fig. 43. Another fruit-body of *C. sterquilinus*, which had shed about one-half

its spores, was crushed up in a mortar, and the contents of the mortar were then filtered through three thicknesses of filter-paper. The filtrate, which was spore-free and brownish, was allowed to stand for 24 hours during which time it darkened in colour. It was then used to write the words shown in Fig. 44. A comparison shows that the writing in Fig. 43 is much darker than that in Fig. 44, thus proving that the intense blackness of the *Coprinus* ink of *C. sterquilinus* is due chiefly to the presence of the spores. Experi-

Written with the
Sporeless Juice of
Coprinus sterquilinus.

FIG. 44.—The words were written with a brush dipped in the juice of an autodigested pileus of *Coprinus sterquilinus*, from which the spores had been removed by filtration. The dark colour of the ink is here due solely to a dark brown pigment produced by the action of one or more oxidising enzymes. Reduced to two-thirds the original size.

ment showed that the darkening of the fluid in which the spores are suspended is an oxidative process and is brought about by an oxidase present in the tissues of the fruit-body before autodigestion takes place.

Coprinus ink was made and used by Bulliard more than one hundred years ago. In his great work, Bulliard¹ illustrated *l'Agaric atramentaire* (*Coprinus atramentarius*) and *l'Agaric typhoïde* (*C. comatus*), and in the description of the latter referred to the brownish ink (*encre bistrée*) as excellent for washes and for pen-and-ink

¹ P. Bulliard, *Herbier de la France*, Paris, 1784, p. 406.

drawings. He boiled the ink and then added some cloves to check the development of moulds.

Boudier,¹ in 1876, wrote the whole of the MS. of his *Notice sur l'encre de Coprin* with the ink of *Coprinus atramentarius*, except the paragraphs relating to *C. comatus*, which he wrote with the ink of *C. comatus*. The MS. was exhibited at a meeting of the Société Botanique de France and the President remarked that the ink which had been used was truly "très-beau noir" and that at first glance it was impossible to tell it from ordinary ink.

Boudier states that the best *Coprinus* for making *Coprinus* ink is *C. atramentarius* (Fig. 45; also Vol. III. Figs. 108-113, pp. 262-269) and that the ink may readily be prepared as fol-



FIG. 45.—*Coprinus atramentarius*, from which *Coprinus* ink can be made. A group of fruit-bodies by a wooden fence. Photographed at Sutton Park, Warwickshire, by the late J. E. Titley. Reduced to about two-sevenths the natural size.

lows. Gather some of the fruit-bodies just before they begin to deliquesce and put them in a glass vessel. The fruit-bodies soon undergo decomposition and a black liquid drips from them. Filter the liquid through cheese-cloth. Often, at first, the ink is somewhat pale: it is then best to let it stand for several days and at the end

¹ E. Boudier, "Notice sur l'encre de Coprin," *Bull. Soc. Bot. de France*, T. XXIII, 1876, pp. 299-302.

of this time to empty away most of the liquid above the spores. To make the ink sufficiently adhesive, add some pieces of gum arabic. To prevent the development of moulds and to improve the odour, add some oil of cloves. Finally, shake up the whole and store it in a stoppered bottle. The ink, before being used, should be shaken.

Boudier calls attention to the fact that *Coprinus* ink, although easily washed away, is not readily altered by chemical reagents such as oxalic acid, is only partially discoloured by chlorine and hypochlorites, and owing to its microscopic characters (presence of spores) is easily recognisable. He suggested that it should be used for documents where forgeries might be attempted.

Boudier further observed that the ink of *Coprinus comatus* (Fig. 46; also Vol. III, Figs. 57 and 58, pp. 148 and 150) is not nearly so good as the ink of *C. atramentarius*, but that it contains so much natural mucilage that it is unnecessary to add any gum to it.

The ink of *Coprinus atramentarius* prepared with the addition of an antiseptic has good keeping qualities. Some of this ink prepared in my laboratory upwards of ten years ago appears to be just as good as when first put into the bottle. *Coprinus* spores do not readily undergo decomposition, and the way in which they are destroyed under natural conditions still remains to be investigated.

Coprinus atramentarius ink is far better than *C. sterquilinus* ink. The chief reason for this difference appears to be that in *C. atramentarius* the spores are very small ($10 \times 5.5 \mu$) and numerous, whereas in *C. sterquilinus* they are very large ($18 \times 10\text{--}11 \mu$) and relatively few.

The Hyphal String in the Stipe of *Coprinus comatus*.—In a stipe of *Coprinus comatus* or *C. sterquilinus* which is elongating there is a soft loose hyphal string which extends through the centre of the cavity from top to bottom. This hyphal string, as formed in a fruit-body of *C. comatus*, is illustrated in the photograph reproduced in Fig. 46. The young stipe is made up of an outer firm cylinder and of an inner softer core. The hyphae making up the core are loosely netted. As the stipe, owing to the enlargement of the firm cylinder, grows in length and diameter, it becomes hollow, and the hyphae of the core become drawn more or less

together to form a hyphal string attached to the pileus-flesh above and the solid base of the stipe below. As the stipe continues to grow in length, the hyphal string is drawn out until it breaks. The hyphal string in the stipe of *C. sterquilinus*, a species closely allied to *C. comatus*, is illustrated in Volume III, Fig. 73 (p. 184).

A Further Remark on the Pileus-flesh of *Coprinus comatus*.—In the fruit-body of *Coprinus comatus* shown in Fig. 46 it may be noticed how well-developed the gills are relatively to the pileus-flesh. No agaric of the Non-Coprinus Type shows so little flesh in combination with such long gills. As the pileus of such a fruit-body as that shown in Fig. 46 expands, the gills, owing to the flesh growing radially more below than above, are gradually turned outwards and upwards through a right angle. It would be mechanically impossible for so small an amount of flesh to accomplish this task were it not for the fact that, as the turning process takes place, owing to the autodigestion of the gills from below upwards the burden to be lifted becomes progressively lighter.

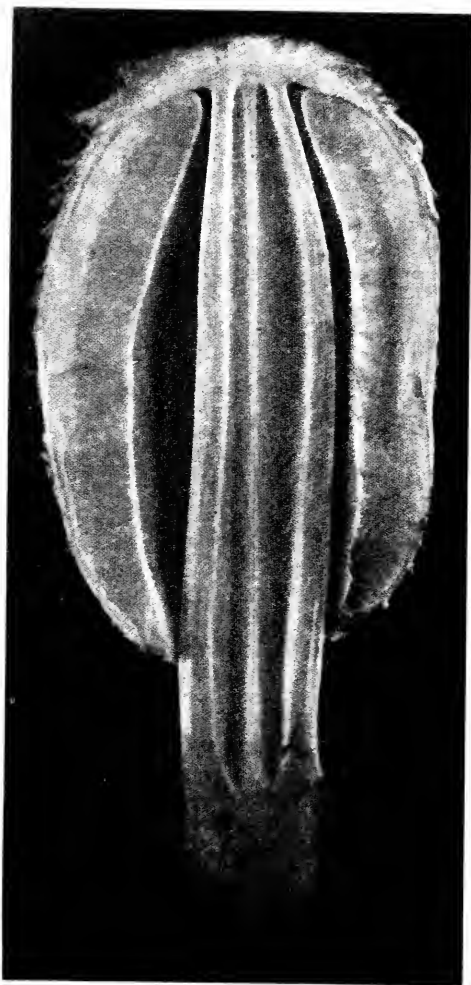


FIG. 46.—*Coprinus comatus*. Vertical section through a fruit-body, to show the soft hyphal string which extends from the top to the bottom of the stipe down the centre of the cavity. The gills are still white as the spores have not yet ripened. Photographed at Winnipeg, 1912. Natural size.

A Giant Fruit-body of *Coprinus comatus*.—In a fruit-body of *Coprinus comatus*, the unexpanded pileus does not usually exceed a height of about 8 cm. nor the fully extended stipe a height of about 25 cm. The largest fruit-body of this species of which there is any record appears to be one collected by Dr. C. W. Dodge of Harvard University. During an expedition made on November 22, 1929, between San Isidro Coronado and San Miguel in San José province, Costa Rica (Central America), he¹ found a fruit-body of *C. comatus* which had an unexpanded pileus of 20 cm. (8 inches) in height and 6 cm. (2·4 inches) in diameter, while the stipe, which was 2 cm. (0·78 inch) in diameter, had already attained a height of 37 cm. (1 foot, 2·6 inches). Since the stipe of *C. comatus* always continues to elongate during the expansion and autodigestion of the pileus, there can be but little doubt that, had the large fruit-body found by Dr. Dodge been left undisturbed, its stipe would have become at least 10 cm. longer and possibly its total length would have exceeded 50 cm. (1 foot, 7·7 inches). The specimen has been deposited by Dr. Dodge in the herbarium of Cryptogamic Botany of Harvard University under the number of 4953.

Excretion of Drops of Water from a Pileus of *Coprinus niveus*.—The excretion of drops of water from the pilei of various Hymenomyces developing under moist conditions has long been observed. It occurs, for example, in *Merulius lacrymans*, *Polyporus squamosus* (at the pores), *Fistulina hepatica* (drops red), and *Coprinus sterquilinus* (Vol. III, Fig. 74, p. 185). An illustration showing drops which have been excreted by the revolute rim of the pileus of a fruit-body of *Coprinus niveus* undergoing autodigestion is shown in the photograph reproduced in Fig. 47. The liquid liberated along the free edge of each gill as a result of autodigestion is being sucked up into the trama by capillarity, and the drops shown in the photograph are due solely to local excretion.

***Coprinus lagopus* growing on Beet Seeds.**—In 1914, Mr. Raymond Finlayson of the Seed Testing Laboratory, Wood Green, London, observed a *Coprinus* coming up on germinating seeds² of Mangel, Beet (*Beta vulgaris*), and Sainfoin (*Onobrychis sativa*), and he sent

¹ C. W. Dodge, *in litt.*, Nov. 24, 1930.

² Mangel and Beet seeds, when sown, are enclosed by a rough pericarp. The *Coprinus* grows on the pericarp.

some of the material to Mr. Carleton Rea. Mr. Rea¹ regarded the fungus as *Coprinus pilosus* Beck, but sent some of the seeds to me with a request for my opinion. The *Coprinus* duly came up on some of the moistened seeds, and from its morphological characters I had no hesitation in concluding that it was identical with the form of *C. lagopus* which occurs very commonly on horse dung both in England and in Canada. A sketch of two rather small fruit-bodies which came up on the seeds is shown in Volume III, Fig. 141, D (p. 320).²



FIG. 47.—*Coprinus niveus*. A fruit-body on horse dung in a dish covered by a bell-jar. Spore-discharge in progress. The edge of the pileus is revolute and rolled. Water drops have been excreted at the edge of the pileus from those parts of the gills which have already shed their spores. Such an excretion only occurs when the air is saturated or almost saturated with water vapour, and the drops may be derived from water liberated and sucked between the trama of the gills during autodigestion. Photographed in the laboratory at Winnipeg. Natural size.

In 1919, Pape³ published a note on the occurrence of a *Coprinus* on germinating Mangel and Beet seeds. His attention had been called to the matter by Dr. Zade of Jena. Pape observed that the fungus grew almost invariably on the seed-balls (pericarps) themselves and he observed nothing to suggest that the fungus is a parasite. He says: "The question of the significance of the fungus in respect to the beet seed upon which it grows must be left open. A lowering of the germinating power of the seed due to the fungus or damage to the young seedling from it has not been observed. The fact that the fungus is most abundant upon seed which we found to have a low

¹ Carleton Rea, *in litt.*

² The occurrence of these fruit-bodies on seeds is referred to in the same volume on p. 308.

³ Pape, "Coprinus auf Rübensamen," *Mitteilungen a. d. Biol. Reichsanst. f. Land- und Forstwirtschaft*, Heft 17, 1919, pp. 13-16, with two illustrations.

germinating power may be due to the fungus finding more nourishment in seeds which have failed to germinate than in those which



FIG. 48.—*Coprinus lagopus* coming up on Sugar-beet seeds sent to the author from Lafayette, Indiana, U.S.A., 1926, by M. W. Gardner. The healthy seeds developed seedlings, and the mycelium in the infected ones produced fruit-bodies. This species is identical with: (1) that used at the University of Manitoba by Irene Mounce, Dorothy Newton, and Hanna in their studies on sex; (2) that described as *C. lagopus* in Vol. III of these *Researches*; and (3) the *Coprinus finetianus* employed by Mlle Beusaude in her classical study of sex in the Hymenomycetes recorded in 1917 and more fully in 1918. Natural size.

produce seedlings.” Pape identified the fungus as *Coprinus nycthemerus* Fr.

In 1926, the *Coprinus* was observed by Mr. W. A. Dillon Weston, of the School of Agriculture at Cambridge, England, coming up on Mangel seeds, and by Mr. M. W. Gardner, of the Agricultural

Experiment Station at Purdue University, Lafayette, U.S.A., coming up on Sugar-beet seeds; and each of these investigators sent me a packet of his infected seeds with a request for an identification of the fungus.

New *Coprinus* fruit-bodies were obtained by the writer, working in association with Miss Dorothy Newton, from (1) the Mangel seeds and (2) the Sugar-beet seeds, by sowing the seeds on sheets of wet filter-paper in a crystallising dish. Some of the seeds germinated normally, whilst others did not germinate at all. On the latter, in both cultures, after a lapse of about three weeks, several *Coprinus* fruit-bodies appeared, and a photograph of one of the cultures showing fruit-bodies and Sugar-beet seedlings is reproduced in Fig. 48. The *Coprinus* on the Mangel seeds was identical in appearance with the *Coprinus* on the Sugar-beet seeds, and both *Coprini* had all the morphological and growth characteristics of *Coprinus lagopus* Fr., as described and illustrated in Volume III of these *Researches*.

Miss Newton and I procured spores from (1) the *Coprinus* on the Mangel seeds, (2) the *Coprinus* on the Sugar-beet seeds, and (3) *Coprinus lagopus* coming up spontaneously on horse-dung balls at Winnipeg and, by a series of mating experiments which are fully described in a special paper,¹ we convinced ourselves that the three *Coprini* are identical. In another paper Hanna,² on the basis of mating experiments, has shown that the *Coprinus lagopus* of Winnipeg, Canada, and the *C. lagopus* of Birmingham, England, are identical.

From the above discussion we are justified in concluding that, as shown by comparative morphology and by mating experiments, the *Coprinus* which comes up on germinating Mangel seeds in England and on germinating Sugar-beet seeds in the United States of America is identical with *Coprinus lagopus* Fr. as it occurs on horse dung in England and Canada and as described and illustrated by the writer in Vol. III of these *Researches*.

¹ A. H. R. Buller and Dorothy Newton, "The Mating Method of Identification of a *Coprinus* growing on Germinating Seeds of Mangel and Sugar-beet," *Annals of Botany*, vol. xli, 1927, pp. 663-670 and Plate XXVIII.

² W. F. Hanna, "The Problem of Sex in *Coprinus lagopus*," *Annals of Botany*, vol. xxxix, 1925, pp. 431-457.

CHAPTER IV

THE GERMINATION OF THE SPORES OF *COPRINUS STERQUILINUS* AND OF OTHER COPROPHILOUS COPRINI

Coprinus sterquilinus and its Spores.—In Nature, Spores of *Coprinus sterquilinus* which Infect Horse Dung have all been previously Swallowed by Horses—Presence of Bacteria Not Required for the Germination of the Spores of *Coprinus sterquilinus*—High Temperature Not Required for Germination—Germination of Spores immediately after their Discharge—Possible Effect of Bacteria on Germination—The Struggle for the Substratum—Remarks on the Germination of the Spores of other Coprini

Coprinus sterquilinus and its Spores.—The production and liberation of spores of *Coprinus sterquilinus* (Fig. 49) was treated of in detail in Vol. III. The fungus is coprophilous and can be found on horse dung in pastures, gardens, and other places. As the pileus expands, spore-discharge begins and, in the course of the few hours of the spore-discharge period, the pileus sheds many millions of spores.

A photomicrograph of some spores of *Coprinus sterquilinus* is reproduced in Fig. 50. The spores fell on to and stuck to a dry glass slide, and they were photographed undisturbed *in situ* in the dry condition. It has been found far easier to obtain a good photograph of *C. sterquilinus* spores when they are dry than when they have been immersed in water under a cover-glass.

Stages in the germination of some spores of *Coprinus sterquilinus* are shown in Fig. 51. The spores were taken from a spore-deposit fourteen months old and were placed in cleared and sterilised horse-dung agar, where they soon germinated. The mycelium shown at F was produced from a spore which had been in the culture medium only 17·5 hours. A germinating spore always emits its germ-tube at its apex and from its germ-pore, and the germ-tube soon begins to branch and to grow away from the spore in all directions. In Fig. 51 the development of vacuoles is shown

in the drawings G–J, and the formation of septa in the drawings I, J, and F.

A cytological study of a young mycelium of *Coprinus sterquilinus*



FIG. 49.—*Coprinus sterquilinus*. Fruit-bodies which came up spontaneously on unsterilised horse dung sent from England. Left: a young fruit-body, which originated in a dark crevice between three dung-balls, still snowy white; a white layer of mycelium covers the front part of the dung-ball. Right: a small fruit-body, which originated at the base of a dung-ball; the blackness of the pileus is due (1) to the black spores on the hymenium of each gill and (2) to a dark-brown pigment in the cells of the hymenium, trama, and pileus-flesh; the blackish tint of the stipe is not due to bruising but to a dark-brown pigment developed in its cells; the pileus is expanding but has not yet begun to shed any of its spores. Natural size.

produced in the course of about 24 hours from a spore in a film of dung-agar on a slide in a moist Petri dish is represented in Fig. 52. The mycelium was fixed in formalin-acetic-alcohol, was stained with iron-alum hæmatoxylin, and was counterstained with light-

green. The germ-tube had issued from the terminal germ-pore of the spore and had developed into a mycelium with coarse radiating hyphae. Not a single septum is to be seen in the whole of the mycelium, and yet the mycelium contains 233 nuclei. The mycelium is therefore very markedly coenocytic. Each nucleus has a thin inconspicuous membrane and a well-stained nucleolus. The mycelium is in the haploid condition, for its nuclei are scattered about

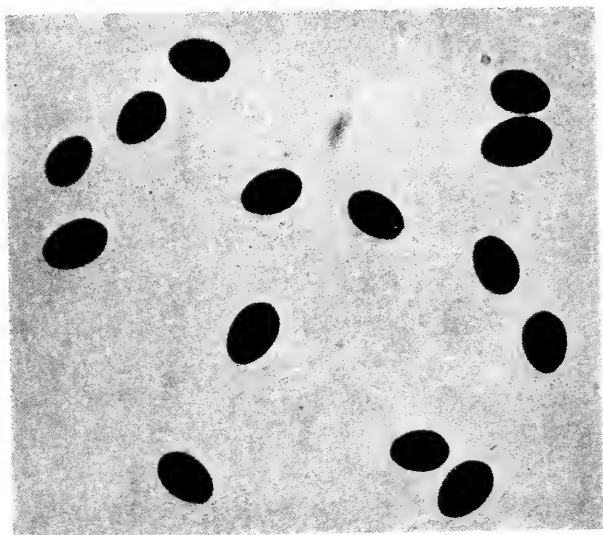


FIG. 50.—*Coprinus sterquilinus*. Spores which had fallen on to a glass slide, photographed dry. Magnification, 500.

in the cytoplasm. In certain places, as at the ends of the hyphae *d* and *e*, the nuclei are in twos; but there can be but little doubt that this is due to active nuclear division and not to the association of the nuclei in conjugate pairs. When a nucleus has just divided, the pair of daughter nuclei must, perforce, at first be close together, although a little later they may wander apart.

In Nature, Spores of *Coprinus sterquilinus* which infect Horse Dung have all been previously Swallowed by Horses.—When spores of *Coprinus sterquilinus* are allowed to fall on sterilised horse dung in the laboratory, infection of the dung rapidly takes place with a subsequent production of normal fruit-bodies.¹ One may therefore

¹ Cf. these *Researches*, vol. iii, 1924, pp. 181–183.

enquire whether the spores liberated by the fruit-bodies of this fungus infect the dung in fields by settling on it directly from the air, or whether they first settle on the grass and are then swallowed by a grazing horse so that they pass down an alimentary canal, become involved in the faeces, and thus come to be embedded in

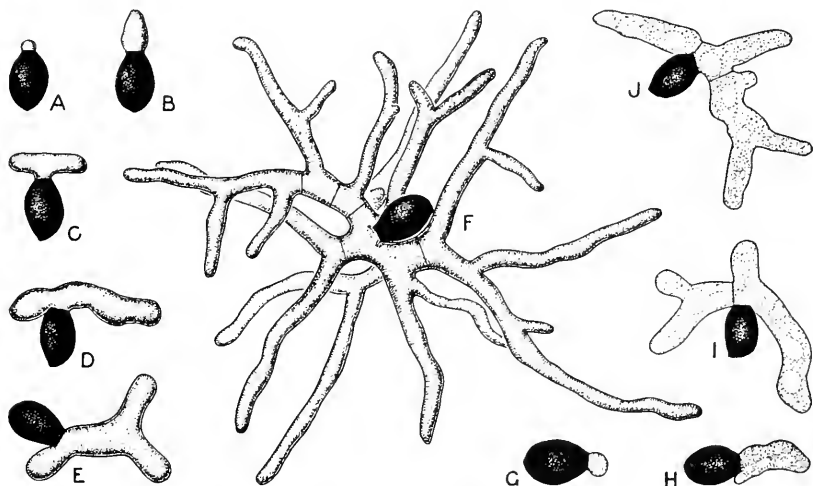


FIG. 51.—*Coprinus sterquilinus*. Germination of spores in absence of bacteria. Spores taken from a spore-deposit 14 months old were placed in hanging drops of cleared and sterilised horse-dung agar, where they soon germinated. The time they had been immersed in the culture medium before being drawn was: A–E, G, and H, 12·5–13·5 hours; I and J, 15 hours; F, 17·5 hours. The germ-tubes and young mycelia are shown in A–F in external view and in G–J in optical section. The germ-tube of each spore has been emitted at the apical germ-pore. The development of vacuoles is seen in G–J. There are no septa in D, E, and H, one septum in I, two in J, and five in F. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 400.

the excreta at the moment when these are extruded and deposited on the ground in the form of dung-balls. It is, of course, conceivable that both methods of infection take place. However, as a result of experiments which will now be described, there seems no reason for doubting that the actual mode of infection is always *via* the alimentary canal of the horse.

Experiment I. Some just-dropped horse-dung balls were obtained from a stable. The surface of the unsterilised balls was then darkened with spores by rubbing it with the gills of a large expanding fruit-body from which spores were being discharged. The balls

were then set in a crystallising dish which was covered with a glass plate and placed on a table in the laboratory. After three days the sporangiophores of *Pilobolus longipes* appeared in the culture in large numbers, and at the end of eleven days the fruit-bodies of *Coprinus curtus* and *C. lagopus* began to come up ; but no sign of *Coprinus sterquilinus* ever showed itself ; its characteristic dense white mycelial pellicle, which is always developed upon the surface of the dung-balls prior to the formation of fruit-bodies, was never produced.

Experiment II. Dung-balls which had been dropped only a few minutes previously were obtained from a stable. A thick three-day-old spore-deposit, collected on white paper, was then moistened with tap-water and rubbed on to the surfaces of the unsterilised dung-balls until these appeared quite black from the innumerable spores thus deposited on them. The dung-balls were then placed in closed crystallising dishes as in the preceding experiment. After a few days the usual flora of Moulds and small species of *Coprinus* made its appearance, but *Coprinus sterquilinus* entirely failed to develop. The non-development of *Coprinus sterquilinus* could not be ascribed to any deficiency in spore vitality ; for the spores were only three days old, and it is certain that dried spores of this fungus retain their power of germination unimpaired for a very considerable number of weeks.

Experiment III. Dung-balls which had been dropped only a few minutes previously were obtained from a stable. The balls were not kept intact as in the previous experiments, but were broken up into fine fragments in a dish. Two thick spore-deposits, which were three days old, were then moistened with tap-water and intimately worked into the unsterilised dung-mass by kneading with the hands. In this way many millions of spores came to be scattered everywhere throughout the dung-mass. The dung-mass was then pressed together and placed in a crystallising dish, as in Experiments I and II. During several subsequent weeks there came up on it the usual succession of Moulds and small Coprini. *Coprinus lagopus*, especially, became very plentiful ; but there was never any sign of *Coprinus sterquilinus*.

When horse dung is sterilised and spores of *Coprinus sterquilinus*

are rubbed upon it either directly from gills or from a recent spore-deposit, the spores germinate and fruit-bodies are subsequently

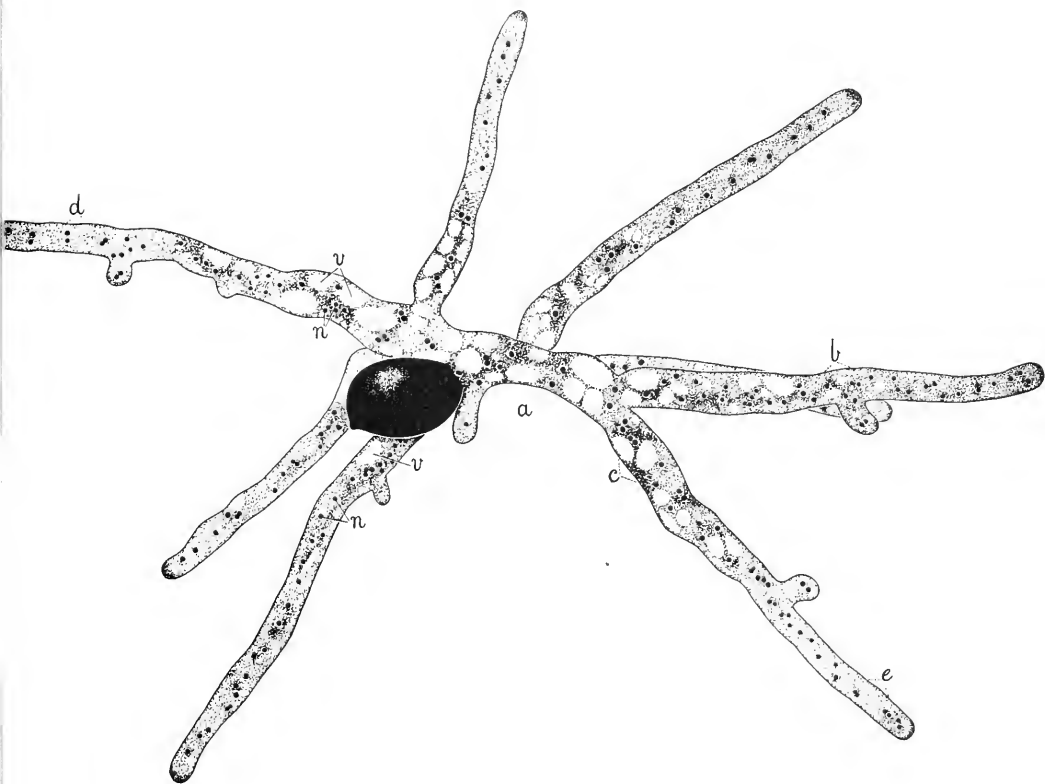


FIG. 52.—*Coprinus sterquilinus*. Camera-lucida drawing of a young mycelium produced in the course of about 24 hours from a spore sown in a sterile culture medium. The spore was placed in a thin film of dung-agar on a slide in a moist Petri dish. The mycelium was fixed in formalin-acetic-alcohol, was stained with iron-alum hæmatoxylin, and was counterstained with light-green. The germ-tube issued from the terminal germ-pore of the black spore shown in the centre of the drawing and it developed into a mycelium with coarse radiating hyphæ, up to the present without a single cross-wall: *a*, at this spot there was a small ungerminated spore which probably prevented the mycelium from sending out a branch in this direction; the hypha *b* almost conceals a hypha beneath it, and a short hypha is entirely concealed by the hypha *c*; *n n*, nuclei with thin inconspicuous membranes and clear contents except for the relatively large and well-stained nucleoli; *v v*, vacuoles in the cytoplasm. The number of the nuclei in the whole mycelium was carefully counted and found to be 233, so that the mycelium which, as yet, is unicellular, is a single coenocyte. The nuclei, as at *n n*, are usually solitary; but, at the ends of certain hyphæ, e.g. *d* and *e*, where nuclear division has evidently just been very active, the nuclei are in pairs: there is no evidence of conjugate nuclear divisions at this stage of development. Winnipeg material. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 850.

produced. The failure of *C. sterquilinus* to develop in the three experiments upon unsterilised horse dung described above cannot be attributed to any want of vitality of the spores. It seems rather to have been due to the fact that the fungus was obliged to compete with other fungi under disadvantageous conditions and was beaten in the struggle. Upon grass settle the spores of many coprophilous fungi, and grass and spores pass down the alimentary canal of the horse together. Probably the initial steps in the germination of the spores are taken in the faeces just before these are extruded. We may therefore suppose that, as soon as dung-balls are deposited on the ground, innumerable spores within the balls begin to form mycelia. Now it is evident that spores of a coprophilous fungus which settled from the air on the surface of a fresh horse-dung ball would have but a poor chance of producing mycelia that could successfully compete with the mycelia produced from spores which had passed down the alimentary canal and were scattered through the substance of the dung-mass. Under the most favourable conditions of culture and temperature (30° C.), in a long series of experiments, it was found that the spores of *C. sterquilinus* do not begin to show signs of germination until 12–24 hours have passed, although within two days between 80 and 90 per cent. of the total number of spores produce germ-tubes. It is clear therefore that, in Experiments I, II, and III described above, the spores of *C. sterquilinus* could produce mycelia only long after their deposition, at a time when, as there is every reason to believe, the spores of other fungi, including Coprini, had already developed an abundance of mycelium which was penetrating the dung-mass in all directions and using up rapidly the most available and the most nutritious food-stuffs. Under these conditions, *Coprinus ephemerus* and *C. lagopus*, together with other fungi, owing to priority of development, seem to have actually suppressed any competition from *Coprinus sterquilinus*.

The experiments and observations recorded above seem to warrant the conclusion that the successful spores of *Coprinus sterquilinus* under natural conditions are those which are swallowed by horses and taken down their alimentary canals, and that any-spores which are not so swallowed, even should they settle on

freshly-deposited horse dung, have practically no chance of ever producing mycelia and of thus giving rise to fruit-bodies. Passage of spores down the alimentary canal of a horse, therefore, appears to be an essential feature in the normal life-history of *Coprinus sterquilinus*.

It is probable that coprophilous Agaricineae in general, e.g. *Panaeoli*, *Strophariae*, other *Coprini*, etc., resemble *Coprinus sterquilinus* in that the spores which germinate and give rise to fruiting mycelia on dung in pastures, etc., are not those which are blown on to the surface of the dung by the wind but are those which have passed down the alimentary canal of some herbivorous animal. So dependent on herbivorous animals have *C. sterquilinus* and other coprophilous Agaricineae become that their geographical distribution is doubtless limited by the geographical distribution of the animals with which they are associated.

Presence of Bacteria Not Required for the Germination of the Spores of *Coprinus sterquilinus*.—In 1915 Miss Baden¹ published a paper in which she asserted that the spores of *Coprinus sterquilinus* germinate (1) only in the presence of bacteria, and (2) only at a temperature above 20° C. A series of experiments carried out by S. G. Churchward and myself in 1915 yielded results which show that both of Miss Baden's conclusions are erroneous.

Churchyard and I found that from 80 to 90 per cent. of the spores of *Coprinus sterquilinus* germinate in hanging drops of the following sterilised solutions: (1) horse-dung decoction, (2) malt extract, (3) bean decoction (*Phaseolus*), (4) Pollock's optimum soil-fungus medium, and (5) nutrient gelatine.

(1) The dung decoction was made as follows. Fresh horse-dung balls were placed in a bag made of cheese-cloth, which was squeezed so that as much of the juice was expressed as possible. The juice was then boiled, to get rid of the coagulable proteins, and filtered. The filtrate was then placed in a flask with a cotton-wool plug and sterilised by heating in a steam steriliser at a temperature of 100° C. for one hour.

(2) To prepare the malt extract, 20 grams of malt were crushed

¹ M. L. Baden, "Observations on the Germination of the Spores of *Coprinus sterquilinus* Fr.," *Annals of Botany*, vol. xxix, 1915, p. 141.

in a mortar and thoroughly mixed with 50 c.c. of distilled water ; and the mixture was allowed to stand for 10 minutes. The extract was then filtered off and placed in a flask with a cotton-wool plug and sterilised by heating in a steam steriliser at a temperature of 100° C. for one hour.

(3) The bean decoction was made from fresh String Beans (*Phaseolus multiflorus*). The bean pods were crushed in a mortar and mixed with about their own weight of distilled water. After filtering, the filtrate was placed in a flask with a cotton-wool plug and sterilised by heating for 45 minutes in a steam steriliser at a temperature of 100° C.

(4) Pollock's formula¹ for his optimum culture medium for a soil fungus is given in gram-molecules as follows : saccharose $\frac{2}{5}$ M., calcium nitrate $\frac{1}{250}$ M., monopotassium phosphate $\frac{1}{10} - \frac{1}{100}$ M., and magnesium sulphate $\frac{1}{1000}$ M.

(5) The nutrient gelatine had the following composition : distilled water 500 c.c., gelatine 50 gm., dextrose 5 gm., peptone 5 gm., Liebig's meat-extract 1.5 gm., and sodium chloride 2.5 gm. The mixture was neutralised with sodium hydroxide, cleared with two eggs, and filtered through cotton-wool. The medium was then placed in a flask with a cotton-wool plug and sterilised by heating at a temperature of 100° C. twice, the first time for 1 hour and the second time, 48 hours later, for 45 minutes.

In making the hanging-drop cultures, all the usual precautions were taken to prevent accidental infection by bacteria, yeasts, or moulds. The apparatus of the glass cells was arranged in most of the experiments in the manner recommended by B. M. Duggar.² A Petri dish was taken and a piece of filter paper was fitted within its base. In this piece of filter paper were made ten circular holes, each one with a diameter just a little larger than that of the glass rings to be employed. Ten glass rings were then set in the ten holes, a cover-glass was laid on each ring, and the cover of the Petri dish applied. The whole was then sterilised in a hot-air oven at a temperature of 160° C. for one hour. A drop of the culture medium

¹ J. B. Pollock, "An optimum culture medium for a soil fungus," *Science*, N.S., vol. 37, 1913, pp. 286-287.

² B. M. Duggar, *Fungus Diseases of Plants*, New York, 1909, pp. 57-59.

to be tested was then placed on the under surface of each of the ten cover-glasses with the help of a sterilised platinum loop ; and each drop was inoculated with spores removed from a pure spore-deposit with a sterilised needle. After the ten rings had all been provided with hanging drops containing spores, a little specially distilled water was placed in the bottom of the Petri dish and the dish cover again applied. The whole apparatus was then placed in the dark. The spore-deposits used for inoculating the drops were collected by placing sterilised glass slides just beneath pilei which were shedding spores in a small damp-chamber ; and they were kept dry in a sterilised box for from 6 to 38 days before being used. Duggar's arrangement for the glass cells has various good features of which ease of sterilisation and the avoidance of either wax or vaseline are the chief. In a few experiments ordinary glass-ring cells were employed. A single ring was attached to a glass slide with paraffin wax, distilled water was placed in the bottom of the cell, and the cover-glass with its hanging drop containing spores was sealed with vaseline. The ring and cover-glass were sterilised with a flame before being used. The drops into which the spores were introduced remained free from bacteria for as long as they were observed, *i.e.* for several days. Had bacteria developed, they would have shown themselves under the microscope in the nutrient gelatine as colonies, and in the liquid media as a cloud of individual particles ; but no traces of these organisms ever appeared in any of the critical experiments.

In one series of experiments, the drop cultures, made in five series with the five different kinds of culture media, were kept in a chamber in which the temperature was maintained at about 30° C. Under these conditions the first signs of germination usually occurred from 23 to 37 hours after the spores had been sown ; and from 80 to 90 per cent. of the spores were found to have germinated at the end of about 48 hours. No bacteria appeared in any of the drops. This series of experiments seems to prove conclusively that the presence of bacteria is not required for the germination of the spores.

Further evidence that the spores will germinate in the absence of bacteria was obtained in the following manner. A series of test-

tubes, 8 inches high and 1·5 inches wide, were half-filled with fresh horse-dung balls and plugged with cotton-wool. The tubes were then sterilised in a steam steriliser by heating them on three successive days for one hour at a temperature of 100° C. Each tube was then inoculated by removing its plug and holding above its mouth, for from 15 to 30 seconds, a pileus of *Coprinus sterquilinus* which was actively shedding spores. The plug was then replaced and the tubes were kept at laboratory temperature. The inoculation was carried out in a large damp-chamber, the air of which was presumably free from bacteria. In the course of a few days the mycelium of the fungus began to develop in each of the tubes and, after a few weeks, fruit-bodies made their appearance and came to maturity. There is no reason to suppose that any bacteria entered the tubes during the process of inoculation or that any living bacteria were left in the dung after its final hour in the steam steriliser. In regard to the latter part of this statement, it may be added that it was found by plating out with nutrient gelatine that, while one application of heat for one hour at 100° C. does not completely sterilise fresh horse-dung balls, sterilisation is accomplished by a second similar application of heat given after an interval of 48 hours.

In the Winnipeg laboratory, passing observations made (1) by Miss Mounce whilst studying the homothallism of *Coprinus sterquilinus* and (2) by Hanna whilst studying the inheritance of spore size in *C. sterquilinus* have completely confirmed the observations of Churchward and the writer¹ recorded above. In the course of their work, Miss Mounce² and Hanna³ made numerous monosporous cultures of *C. sterquilinus*, and the germination of the spores in the culture medium free from bacteria and other organisms was a *sine qua non* for the success of their experiments. Hanna, employing the dry-needle method, sowed 433 spores of various

¹ The experiments on *Coprinus sterquilinus* made by Churchward and the writer, here recorded, were completed in 1915; but, owing to the war, their publication has been delayed until now.

² Irene Mounce, "Homothallism and the Production of Fruit-bodies by Monosporous Mycelia in the Genus *Coprinus*," *Trans. Brit. Myc. Soc.*, vol. vii, 1921, pp. 198-217; also "Homothallism and Heterothallism in the Genus *Coprinus*," *ibid.*, 1922, pp. 256-269.

³ W. F. Hanna, "The Inheritance of Spore Size in *Coprinus sterquilinus*," *Trans. Brit. Myc. Soc.*, vol. xi, 1926, pp. 224-225.

sizes, selected from fifty different fruit-bodies, singly in sterile hanging drops of nutrient gelatine (containing dextrose, peptone, beef-extract, and sodium chloride), with the result that 101, or 23·3 per cent., germinated.

High Temperature Not Required for Germination.—In another series of experiments, made to test Miss Baden's assertion that the spores of *Coprinus sterquilinus* will not germinate at all below a temperature of 20° C., hanging drops, similar to those already described, were made for all the five kinds of media ; and then the culture apparatus was set in a refrigerator in which the temperature varied from 5° to 10° C. Under these conditions, the first signs of germination usually appeared about 36 hours after the spores had been sown ; but, in the end, from 80 to 90 per cent. of the total number of spores germinated, just as in the experiments made at 30° C. This successful series of experiments, carried out at temperatures far below 20° C., seems to prove conclusively that Miss Baden's assertion was based on an insufficient number of experiments. In such a matter as the one under discussion, a series of positive results by far out-weighs in importance a large number of negative ones.

Germination of Spores immediately after their Discharge.—According to Miss Baden,¹ the spores of *Coprinus sterquilinus* do not mature until about three weeks after they have been shed but, if dried for two days at 40° C., they germinate at once. She therefore supposed that, under natural conditions, the germination of the spores in horse dung is retarded until the substratum has become fairly dry and such fungi as *Mucor* have disappeared. In the course of many years I have made numerous pure cultures of *C. sterquilinus* by inoculating sterilised horse dung with spores which fell on to the medium directly from the gills ; and Hanna,² in the course of his work, could observe no difference between the germination of spores which had just been shed and of spores which had been kept for several weeks. In one of Hanna's experiments ten spores which had just been shed were placed in as many hanging-drops of sterile nutrient gelatine, with the result that, within 24 hours, all of them germinated. There can be no doubt,

¹ M. L. Baden, *loc. cit.*, pp. 135-142.

² W. F. Hanna, *loc. cit.*, p. 225.

therefore, that Miss Baden's conclusions concerning the maturation of the spores of *C. sterquilinus* are erroneous.

Possible Effect of Bacteria on Germination.—While, as shown above, the presence of bacteria is not necessary for the germination of the spores of *Coprinus sterquilinus*, it is yet possible that, under natural conditions in pastures, the germination of the spores, which always takes place in the presence of bacteria, may be affected by bacterial products of metabolism ; but the worth of this suggestion remains to be tested by actual experiment. Spores, on passing down the alimentary canal of a horse, are immersed in a medium which is raised to a high temperature, are bathed for many hours by the strong enzymes with which the horse digests its food and, by the time that they reach the rectum, become surrounded with highly nutrient food substances. Under these conditions of temperature, enzymes, and food substances, although the oxygen supply may be reduced to a minimum, it may well be that the spores undergo those preliminary changes which are necessary for germination, so that the production of germ-tubes may begin almost immediately the faeces are extruded as dung-balls. This seems to be all the more probable, since early germination in freshly-dropped horse-dung balls must, undoubtedly, be an important factor for success in the intense struggle for possession of the substratum which goes on among the competing fungi.

The Struggle for the Substratum.—As bearing on the problem of the struggle for the substratum, we may remind ourselves of the three experiments recorded in the first Section of this Chapter. It was shown that, when freshly-dropped horse-dung balls were supplied with millions of spores of *Coprinus sterquilinus*, this fungus did not succeed in developing any fruit-bodies : it was beaten in the struggle by other competing species of fungi ; it entered the contest under a disadvantageous condition, namely, delayed germination of its spores. In a further experiment this condition was reversed. Pieces of horse dung, overspread with the vigorously growing mycelium of *C. sterquilinus*, were placed upon some just-dropped dung-balls, with the result that the mycelium spread rapidly over the balls, and, in the end, gave rise to fruit-bodies. Artificial priority of development had been given to *C. sterquilinus*

and, in consequence, this fungus succeeded in the competition with the other fungi in the substratum. The mycelium spread superficially at the rate of between 0·5 and 1 cm. *per diem*, but nevertheless did not succeed in spreading over the balls rapidly enough to prevent entirely the local development of some other species of Coprini which gave rise to fruit-bodies in the same culture. The difference in the result of adding spores and of adding mycelium to fresh dung-balls goes far to demonstrate the importance of priority of germination in the struggle for the substratum. The chemical and physical battles which are waged as matters of life and death between the numerous contestants in every horse-dung ball deserve much more attention than they have hitherto received, and offer a fine field for future investigation.

Remarks on the Germination of the Spores of other Coprini.—In the genus *Coprinus*, germination of the spores in sterilised media has been observed, among others, for the following species :

<i>C. sterquilinus</i> ,	<i>C. lagopus</i> ,
<i>C. comatus</i> ,	<i>C. stercorarius</i> ,
<i>C. Rostrupianus</i> ,	<i>C. ephemerus</i> ,
<i>C. niveus</i> ,	<i>C. curtus</i> ,
<i>C. macrorrhizus</i> ,	<i>C. plicatilis</i> .

Up to the present, therefore, there is no reason to suppose that the presence of bacteria is a necessary condition for the germination of the spores of any species of *Coprinus*. However, whether the metabolic products of bacteria either hinder or help the germination of the spores of any particular species of *Coprinus*, or do neither, can only be decided by carefully-made comparative experiments.

CHAPTER V

AN ANALYSIS OF THE FACTORS CONCERNED IN THE MECHANICAL FIXATION OF THE FRUIT-BODY OF *COPRINUS STERQUILINUS* IN ITS SUBSTRATUM

Introduction—Fruit-body Fixation in certain Polyporaceae and Agaricineae—*Coprinus sterquilinus*—The Mycelium at the Surface of the Substratum—The Mycelial Strands within the Substratum—The Rudiments of Fruit-bodies—Light inhibits the Growth of Rudimentary Fruit-bodies—The Morphogenic Effect of Light on Developing Fruit-bodies—The Relations of the Fruit-body with Gravity—The Upward Pressure of the Stipe—The Heliotropic Effect of Light—The Mechanical Fixation of the Fruit-body—Conclusion

Introduction.—The hymenomycetous fruit-body, as we have seen, functions successfully as an organ for the production and liberation of spores only when it is formed in the air and is there *held in one fixed position*. Therefore the ways and means by which Hymenomycetes solve the problem of mechanical fixation for their fruit-bodies is well worth enquiry. The factors which enter into fixation are not always the same but may differ in different species; and, as evidence thereof, a brief description of the mode of fixation of the fruit-bodies of a few of the larger Polyporaceae and Agaricineae will be given in the next Section. The rest of the Chapter will be devoted to a full discussion of the various factors which enter into the fixation of the fruit-bodies of a large coprophilous fungus, namely, *Coprinus sterquilinus*.

Fruit-body Fixation in certain Polyporaceae and Agaricineae.—*Fomes fomentarius* has large hoof-like fruit-bodies which, in Canada, are often seen on the standing trunks of the Paper Birch (Vol. II, Figs. 36 and 37, pp. 106 and 109). Each fruit-body is attached to a tree-trunk by its whole surface of contact, as may be seen by a study of the back of the fruit-body shown in Fig. 53 (also cf. Volume II, Fig. 39, p. 113). The fixation of a large, several-

year-old fruit-body is due in part to the mycelial hyphae, which at first break through a relatively small hole in the bark and give rise to the fruit-body, but chiefly to the fruit-body itself; for this, as it develops, flattens itself out over the uninjured bark and attaches itself very firmly to the bark by means of its adhesive hyphae.



FIG. 53.—*Fomes fomentarius*. The back of a fruit-body, two years old, which grew on a Birch tree near the Lake of the Woods, central Canada. To show the mode of attachment of the fruit-body to the tree. The mycelium burst through the bark only in the area *a*. The fruit-body began its development at *a* and then grew downwards, tending to take a conical form but being prevented from doing so by the presence of the tree-trunk against which it came to press. It became so firmly adherent to the tree-trunk that when it was forcibly broken away therefrom, as shown at *b*, it carried with it a thin outer sheet of the birch-bark (cork) which is easily recognisable by the characteristic lenticels. Natural size.

Other *Fomes* species, *e.g.* *F. officinalis* and *F. applanatus*, attach themselves to tree-trunks in a similar manner.

In many terrestrial Agaricineae, *e.g.* *Amanita rubescens*, *Lepiota procera*, *Psalliota campestris*, *Paxillus involutus*, and *Coprinus picaceus*, a fruit-body, at first, is a minute rudiment produced upon a dense mycelium which permeates leaf-mould or earth rich in organic remains. This rudiment is formed at or near the surface of the ground. As it develops, the stipe becomes more or less

conically pointed or obtusely convex below and, at the same time, thicker. The result is that the base of the stipe comes to press against the surrounding leaf-mould or soil which returns the pressure and, in so doing, gives the stipe mechanical support. Furthermore, the outer hyphae of the base of the stipe penetrate the substratum wherever contact has been made and anchor the stipe to the leaf-mould, etc. Thus the attachment of the base of the stipe to the substratum becomes so extensive and so firm that the fruit-body, when full-grown, is supported by the stipe in one fixed position (*cf.* for *Psalliota campestris* Vol. I, Fig. 76, p. 218 and for *Coprinus picaceus* Vol. III, Fig. 129, p. 297). That terrestrial fruit-bodies take a strong hold on their substratum is proved by the fact that, when one pulls one of them up, one invariably observes leaves, soil particles, or cinders, etc., clinging to the base of the stipe.

In *Collybia radicata* the fruit-body arises as a tiny rudiment on the surface of a buried root of a Beech, Oak, or some other tree. By intercalary growth at the base of the stipe there is formed a rod-like *pseudorhiza* or "rooting base" which gradually pushes the rudimentary pileus and rudimentary stipe-shaft up to the surface of the ground, where they expand and form a large aerial fruit-body. The *pseudorhiza*, as it grows in length and approaches the surface of the ground, gradually thickens and becomes mechanically stronger. Finally, its thickest part is continuous with the base of the aerial stipe-shaft. The fully-developed *pseudorhiza* is held fast in a vertical position by pressure from the surrounding soil and, in turn, the *pseudorhiza* supports the aerial stipe-shaft and pileus. Thus the fixation of a fruit-body of *Collybia radicata* by its *pseudorhiza* is comparable with the fixation of a herbaceous flowering plant, such as a Lupin or a Dandelion, by its tap-root. In respect to the manner in which their fruit-bodies are fixed in the ground, the other agarics with stipes ending in a "rooting base," *e.g.* *Collybia longipes*, *Pholiota radicata*, and *Coprinus macrorrhizus*, resemble *Collybia radicata*.

Coprinus sterquilinus.—*Coprinus sterquilinus* produces its large fruit-bodies (Figs. 54, 55, and 56) in pastures on horse dung. This substratum is made up of more or less ball-like masses irregularly heaped together. One may therefore ask: by what means do the

fruit-bodies of *C. sterquilinus* become fixed in the dung? An attempt to answer this question will be made in what follows. The enquiry was prompted in the first instance by the discovery that, in artificial horse-dung cultures well exposed to the light, the fruit-bodies were never attached to the tops of the dung-balls, but always to their under sides in dark crevices at or near the bottom of the crystallising dish. The various factors which are concerned with the fixation of *C. sterquilinus* fruit-bodies in their position in the substratum will first be considered separately and in detail, and then, in a description of the fixation process, an attempt will be made to correlate them.

The Mycelium at the Surface of its Substratum. — The mycelium of *Coprinus*

sterquilinus at the surface of its horse-dung substratum has various functions to perform, one of which is to aid in the fixation of the fruit-body. To substantiate this statement, it is necessary to describe and discuss the development of the mycelium as observed in the laboratory.

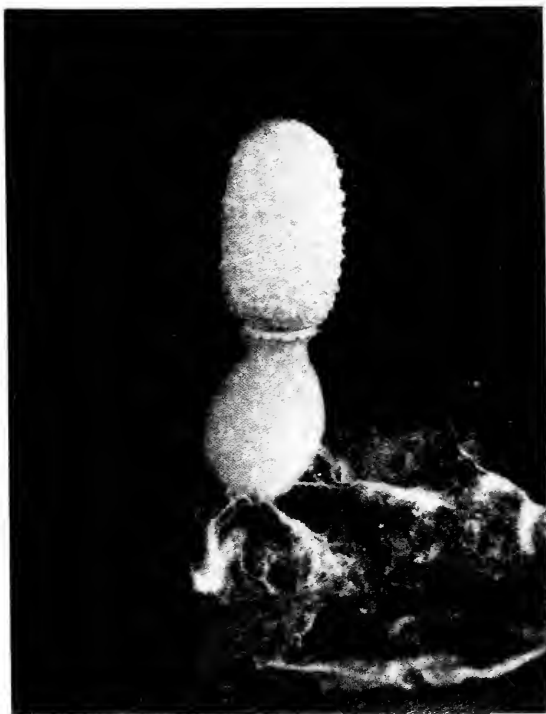


FIG. 54.—*Coprinus sterquilinus*. A young fruit-body coming up on a horse-dung ball in a laboratory dung culture. It originated in a dark crevice between three dung-balls, two of which have been removed. Note the mycelium growing over the surface of the dung-ball and the mycelial strands which assist in attaching the fruit-body to its substratum. The young pileus was soon pushed up into the light. Hence the elongation of the stipe-base was soon inhibited by the light and is relatively short. Natural size.

A few days after the spores of *Coprinus sterquilinus* have been sown upon sterilised horse dung in a dish-culture, like that shown in Fig. 55, the mycelium can be seen spreading rapidly over the substratum in a radial direction from the points of germination. Its radial rate of growth at a temperature of about 25° C. in one culture was observed to be 4 cm. in 5 days or, upon the average, 0·8 cm. per day. Whilst this rapid surface-growth is taking place, the mycelium is also penetrating into the interior of the dung-balls and, about two weeks after the spores have germinated, hyphae are to be found in every part of each ball. The rate of penetration of the mycelium directly through a dung-ball is much slower than the rate of growth upon the ball's exterior, a fact which finds its ready explanation in the difference between the obstacles to be overcome—in the one case hard interlacing straws, etc., and in the other the air or a film of liquid.

A mycelium produced from spores which have germinated on the top of a dung-ball can grow down the sides of the ball and arrive at the bottom of the culture-dish long before any hyphae have succeeded in penetrating directly through the ball from top to bottom. There is no doubt that the hyphae which are produced at the surface of a dung-ball, some of which are in actual contact with its substance and others of which project aerially to a distance of a few mm., are of no small importance in facilitating the attack of the mycelium as a whole upon its substratum. Granted that only part of a dung-ball under natural conditions has been originally infected with the mycelium, then, by means of surface growth, new radial lines of infection may soon be brought into existence. Granted that one ball only of several in a dung-mass has been infected in the first place, then, by surface growth, the hyphae may soon reach the other balls. In both instances the substratum would be occupied much more slowly were its infection confined to the penetrating hyphae and were surface hyphae absent. Owing to the severe competition for the substratum which takes place between coprophilous fungi under natural conditions, slowness of extension of the mycelium—other things being equal—is a fatal handicap, and therefore the development of rapidly-growing surface hyphae which hasten the occupation of new territory is highly

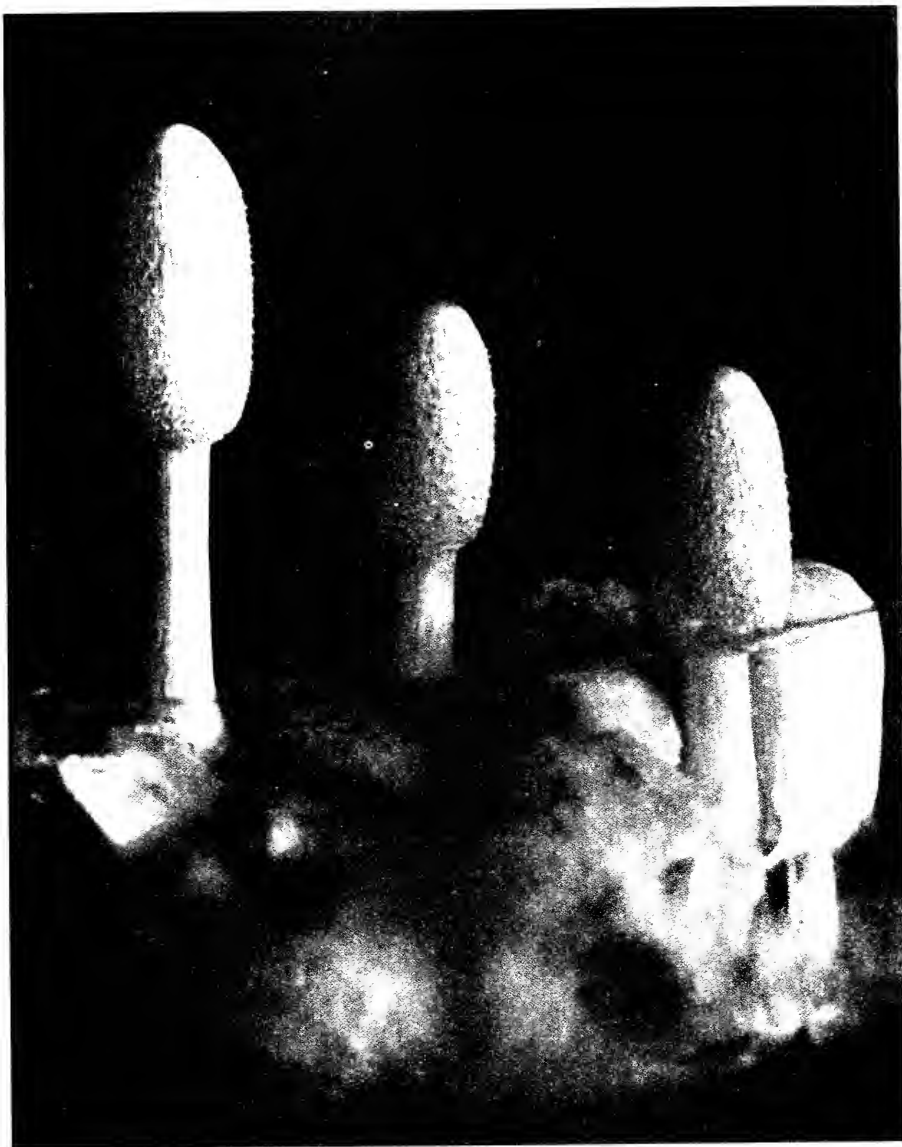


FIG. 55.—*Coprinus sterquilinus*. A group of maturing fruit-bodies coming up on sterilised horse dung in a large crystallising dish. A thin and characteristic white layer of mycelium covers each dung-ball. The fruit-bodies originated in dark clefts between the dung-balls at or near the bottom of the dish. The pilei and stipes are still white because they have not yet begun to develop a brown pigment in the vacuoles of their cells, and each pileus is carrying upwards at its base a ring of tissue which, when the pileus begins to expand, will be dropped on to the stipe as an annulus. Natural size.



advantageous. In *Coprinus sterquilinus* it is particularly important that the mycelium should spread itself as quickly as possible in a dung-ball mass, for a single fruit-body is so large that its development may exhaust the mycelium in from one to three balls. A mycelium which does not succeed in occupying at least a certain minimum volume of its substratum, as a rule at least one dung-ball, remains sterile and does not develop the reproductive apparatus. For this reason it is not surprising to find that the mycelium of *Coprinus sterquilinus* has a particularly rapid rate of surface growth over its substratum, much more rapid than that of the mycelia of smaller Coprini with which it so frequently has to compete. It may be added that the surface growth observed in sterilised cultures also takes place in unsterilised horse-dung balls which have become naturally infected, and which have subsequently been placed under moist conditions in the laboratory. From such material I have been in the habit of procuring the fungus in the first place, and therefore have often had occasion to watch for the first signs of its appearance. These first signs are always the rapidly-growing surface hyphae.

In a dish-culture containing sterilised horse-dung balls inoculated above with spores it invariably happens that the mycelium, on arriving at the base of the balls as a result of superficial growth, continues to grow for some centimetres over the bottom of the dish in the film of moisture there present. Under natural con-

FIG. 56.—*Coprinus sterquilinus*. A mature fruit-body coming up from the base of a horse-dung ball in a pure culture in a large crystallising dish. A thin and characteristic white layer of mycelium coats each dung-ball. The fruit-body is held in its upright position: in part because the lower end of its stipe is attached to the under side of a dung-ball by hyphal strings and the layer of mycelium already mentioned; in part because the base of its stipe rests on the bottom of the dish; in part because its stipe, a little way above its base, is in lateral contact with two dung-balls, and in part because the centre of gravity of the whole fruit-body is almost vertically above the centre of its base. The pileus is still expanding. Its edges have become torn into rays which are becoming revolute. The middle of the spore-discharge period has come, and many millions of spores are being liberated. The pileus is black because a dark-brown pigment is present in the cells of the pileus-flesh, the trama, and the hymenium and because the hymenium on every gill bears jet-black spores. The blackness of the upper portion of the stipe is due to the presence in the cells of the same dark-brown pigment that occurs in the pileus, and not to the deposition of spores. A spore-deposit has already darkened the upper edge of the dish on the left and the layer of mycelium on the tops of dung-balls in the back-ground. Reduced to about two-thirds the natural size.

ditions, this extension of the mycelium beyond its nutrient substratum doubtless often serves to bridge the gap which may exist between one dung-ball and one or more of its fellows as they lie upon the ground in fields, etc. : it may permit the mycelium in one ball to infect another ball by growing to it *via* the surface of the soil or grass-stems. The aerial hyphae which project from the surface of a ball to a distance of a few millimetres may be of advantage in extending the mycelium across air-gaps between nearly touching balls. In dish-cultures I have often noticed the spread of the mycelium from ball to ball in this way. When this has occurred, the aerial bridging hyphae finally become reduced to a few thick mycelial strands. These strands are channels of conduction, and make it possible for a fruit-body which has arisen on one ball to drain the mycelial contents of several balls, and thus to obtain the full quota of materials necessary for its construction.¹

The surface mycelium, when it first makes its appearance at the surface of dung, consists wholly of loose hyphae ; but, after a few days of growth, it forms a dense white membranous covering which adheres closely to the exterior of each infected dung-ball (Figs. 56 and 58) ; also Vol. III, Fig. 71, p. 182). Whilst this change is going on, the older aerial hyphae disappear, and it may be that their contents are withdrawn to be used up by the hyphae forming the membrane. The membrane, as a rule, is not uniform in texture but comes to contain, or even to be chiefly composed of, conducting strands (Fig. 54, p. 95). Along these strands, doubtless, some of

¹ The surface hyphae of other saprophytes, such as Moulds and wood-destroying Hymenomycetes, function in a manner similar to those of *Coprinus sterquilinus*, i.e. by hastening the occupation of the substratum when continuous and by bridging gaps between portions of it when discontinuous. In this connexion the case of *Merulius lacrymans*, the Dry-Rot Fungus, may be cited. Often, in damp cellars in England, the mycelium of this fungus grows out from a wooden beam on to the brick wall and spreads over the wall for a distance of several inches or feet. In so doing, it may encounter other pieces of wood which it then proceeds to infect. Once, in the cellar of a private house at Edgbaston, Birmingham, I observed that the mycelium had spread from a beam downwards over a perfectly bare white-washed wall for a distance of three feet (*cf.* these *Researches*, vol. iii, 1924, pp. 41-42, Figs. 23 and 24). There can be no doubt that, for *Merulius lacrymans*, the surface growth of the mycelium on non-nutrient substances, which gives the fungus the advantage of extending its area of attack, contributes in a marked degree to the destruction of the wood-work in houses.

the contents of the mycelium as a whole pass to the fruit-body when this begins its development.

The surface hyphae also produce the fruit-body rudiments, each of which presumably originates from one single hypha in the manner described by Brefeld¹ for *Coprinus stercorearius* and *C. lagopus* (*vide infra*, Figs. 93-95). Numerous observations have taught me that a fruit-body rudiment never begins its development within the substance of a dung-ball but always at the surface.

The base of the stipe of a fruit-body of *Coprinus sterquilinus* is always connected with one or more strands of the superficial mycelial membrane (Fig. 54, p. 95); and it appears, therefore, that this membrane plays a part in attaching the fruit-body to its substratum. In *Panaeolus campanulatus* we have a parallel phenomenon, but in that fungus the attaching membrane is not so obvious and is gelatinous.²

To sum up the foregoing discussion, it may be said that the mycelium of *Coprinus sterquilinus* at the surface of a dung-ball functions in the following ways: (1) it hastens the occupation of a dung-ball by rapidly growing around the ball's surface and by thus providing new points of infection for radial penetration; (2) it spreads the infection from one dung-ball to another directly when they are in contact, and indirectly when they are slightly separated, by growing over the surface of the intervening non-nutrient substratum or through the air; (3) it forms a membrane consisting largely of conducting strands which are of importance for transmitting food materials to the growing fruit-body; (4) it gives rise to a fruit-body; and (5) it serves to attach the base of the stipe to the substratum and thus assists in the mechanical fixation of the fruit-body.

The Mycelial Strands within the Substratum.—The hyphae in the interior of a dung-ball are chiefly concerned with attacking the food substances there present, and at the same time adding to their number and total volume. Finally, after they have become united into a three-dimensional network,³ they empty themselves by giving

¹ O. Brefeld, *Untersuchungen über Pilze*, Heft III, 1877, Taf. I and Taf. VI.

² Cf. these *Researches*, vol. ii, 1922, p. 250, Fig. 87.

³ *Vide infra*, Part II, chap. i.

up their contents to the fruit-body developing on the exterior of the dung-ball. Toward the end of their vegetative career, they tend to form conducting strands, for a few such strands passing from the interior of the dung-ball are attached to the base of the

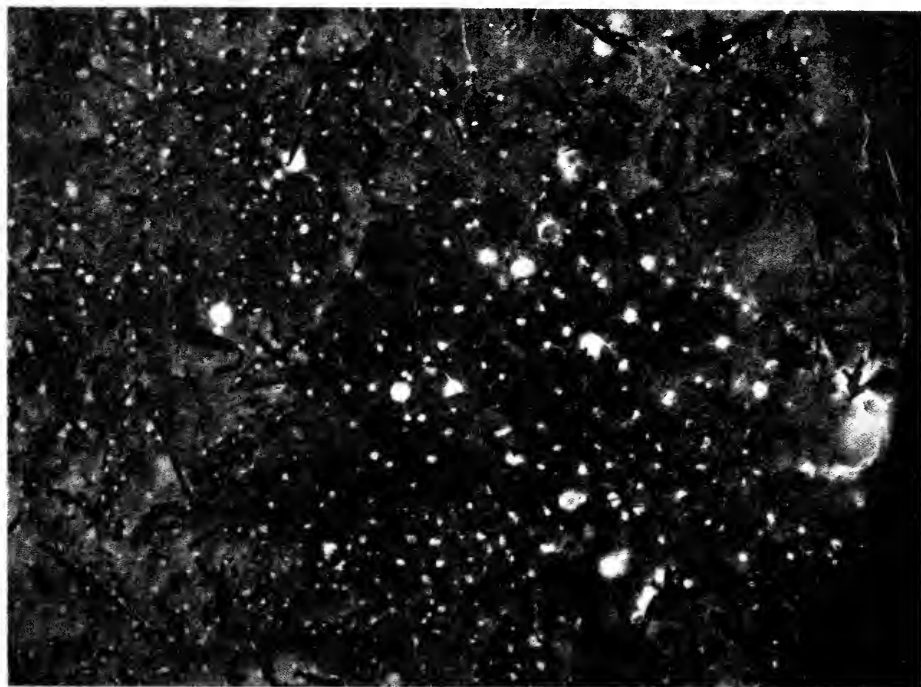


FIG. 57.—Photograph taken through the bottom of a glass dish containing horse dung in which the mycelium of *Coprinus sterquilinus* was growing. The mycelium has produced numerous fruit-body rudiments, one of which (on the extreme right) is developing into a perfect fruit-body. Natural size.

stipe of each fruit-body (Vol. III, Fig. 74, p. 185). These internal strands, like those at the surface of the substratum, assist in the mechanical fixation of the fruit-body.

The Rudiments of Fruit-bodies.—About fourteen days after the spores have been sown in a large crystallising-dish culture, tiny rudiments of fruit-bodies, no larger than a pin's head, begin to appear at numerous points all over the surface of the dung-balls (*cf.* Fig. 57). In a single culture several hundreds of such rudiments begin their development ; but, in the end, only some five or six of

them actually grow up into mature fruit-bodies. The rudiments which are destined to produce spore-bearing fruit-bodies usually arise at or near the bottom of the culture-dish and are separated from one another by intervals of a few inches. They draw to themselves the contents of the mycelium, each one in this respect having its own sphere of influence ; and, apparently by this very simple means, they cause the inhibition of growth of the remaining much more numerous rudiments, so that the latter soon cease their development, wither, and die.¹ Perhaps these aborted rudiments, which at the best usually do not exceed a length of about 3 mm., give up their contents to their more fortunate companions, but the value of this suggestion can be decided only by exact investigation.

We have seen that of the numerous rudiments which begin their development only a small fraction ultimately give rise to mature fruit-bodies. What biological significance may be ascribed to this fact ? We can here give an answer similar to that which was given when a like question arose in regard to the supernumerary rudiments of the Mushroom.² The mycelium produces numerous rudiments at the surface of its substratum ; but only those develop which are in the most favourable positions in respect to the mycelium as a whole and to certain external conditions. The more rudiments there are, the more likely it is that some of them will have originated in positions highly favourable for further growth ; and these few alone complete their development. The supernumerary rudiments, which have all originated in the less favourable places, represent the margin of safety required to secure success in reproduction. The fungus, as it were, proceeds by the method of trial and error : the rudiments which become aborted represent the errors, while those which develop into spore-bearing fruit-bodies represent the

¹ Jacques Loeb (*Regeneration*, New York, 1924) gives a similar explanation for the growth of certain groups of bud-anlagen rather than others, on a leaf of *Bryophyllum calycinum*. According to him, the inhibitory action of the growth of one group of bud-anlagen on other neighbouring groups is due (1) to the fact that sap and nutrients flow to the group that begins growth first, and (2) to the fact that the continuation of the flow to the growing organs prevents the bud-anlagen in other groups from obtaining the sap and nutrients necessary to initiate their growth.

² Cf. these *Researches*, vol. ii, 1922, pp. 398-399.

successes. The development of fruit-bodies in the Hymenomycetes generally is accompanied by this phenomenon. In the next Section some of the factors which decide which of the many rudiments shall continue their development will be considered, and it will be shown that *light* is one of the most important of them.

Light inhibits the Growth of Rudimentary Fruit-bodies.—Large crystallising-dish cultures, covered with glass plates, were exposed to daylight upon a laboratory table. As more and more cultures were watched, it was noticed that the stipes of fully expanded fruit-bodies were usually in contact with the bottom of the dishes (Figs. 58 and 59). Further observations showed that, although numerous tiny fruit-body rudiments came into existence all over the free surfaces of the dung-balls, the particular rudiments which escaped abortion and ultimately developed into mature fruit-bodies always had their origin *at or near the bases of the balls*. Now this position of origin for the rudiments destined to complete their development is, as we shall see later on, a most important factor in securing the mechanical fixation of the full-grown fruit-bodies, which are organs of considerable height and weight. It is therefore of interest to ask the following question: why do those rudiments which happen to arise on the under sides of the dung-balls, and those only, continue their development while all the others become aborted? By a series of experiments which will now be described, I have convinced myself that the answer is to be found in the action of an external agent, not gravity or moisture, but *light*: those rudiments which arise in well-lighted positions are inhibited by the light from further growth, while those which arise in darkness or dimly-lighted positions are not so inhibited.

The critical experimental cultures were four in number and were arranged as follows:

- Culture A. Dung-balls lighted from above and darkened below.
- Culture B. Dung-balls lighted from below and darkened above.
- Culture C. Dung-balls kept in total darkness.
- Culture D. Dung-balls lighted all over their surfaces.

The cultures were prepared for the experiments in the following manner. Fresh dung-balls were fetched from a stable. For the

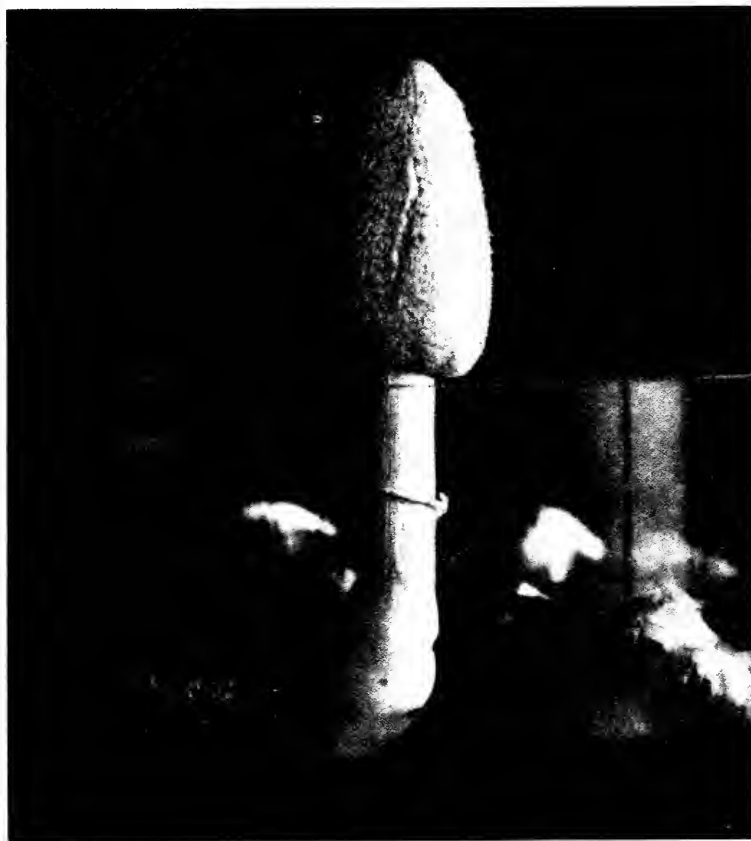


FIG. 58.—*Coprinus sterquilinus*. To show the position of the base of a fruit-body in relation to horse-dung balls in a top-lighted pure culture. A fruit-body arose in a dark crevice below a dung-ball and began to push upwards. When 2 cm. high, its pileus was removed by cutting the stipe across just below the pileus and then a new pileus, cut off from another fruit-body in the same way, was substituted for the first (cf. Fig. 25, A-D, p. 79 in Vol. II). The graft attached itself to the stock and grew vigorously as indicated by the photograph. The compound fruit-body, which has grown in the same manner as any ordinary fruit-body, is well fixed to its substratum owing to pressure by the dish and the base of the dung-ball under which it originated. Winnipeg material. Natural size.

first three cultures large dishes were employed (6–7 cm. high and 18–21 cm. wide). In these the balls were placed and packed closely together so that the spaces between them where they came into

contact were practically obliterated. For the fourth and last culture a smaller dish was found more suitable. In it were placed only three dung-balls, and these were slightly separated from one another so that they might be lighted all over their surfaces. All the dishes were covered by glass plates and then sterilised in a

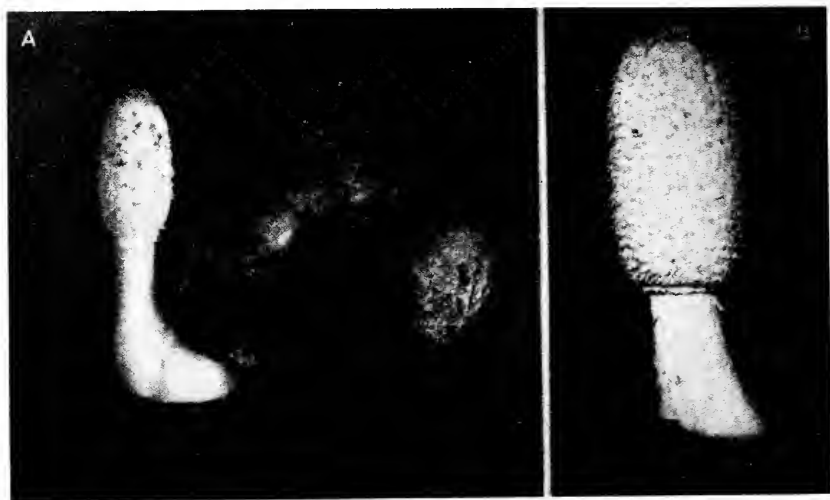


FIG. 59.—*Coprinus sterquilinus*. Fruit-bodies which have arisen in cultures lighted from above and darkened below. A, a horse-dung ball and the fruit-body attached to it, removed from among other balls in the culture dish and photographed in lateral aspect. The fruit-body developed from a rudiment which had its origin in the dark at the base of the dung-ball. B, another fruit-body, photographed *in situ* in Culture A. It has arisen from a rudiment which came into existence in a darkened crevice beneath and between two dung-balls. It has a short, solid, somewhat bulbous stipe-base and a longer hollow stipe-shaft, and the pileus is well advanced. Cf. Fig. 61 which shows fruit-bodies of the same age grown entirely in the dark. Photographed 23 days after the culture was started with mycelium. Natural size.

steam steriliser by heating to 100° C. twice or thrice on successive days, an hour each time. Inoculation was effected by holding a pileus shedding spores over the dung for about half a minute or by transferring pieces of mycelium from other pure cultures.

Cultures A and B. Set up simultaneously. After inoculation, the dish of A was covered at the bottom (outside) with black paper and was then placed on a table at a window, so that it was *lighted* by daylight *from above* and at the sides. The dish of B, on the other hand, was covered with black paper above and at the sides,

and was *lighted* by daylight only *from below*. The lighting was accomplished by setting the crystallising dish on a glass plate supported by an inverted iron tripod at a window and by then reflecting light upwards by means of a mirror placed beneath.

In the top-lighted Culture A, many tiny rudiments of fruit-bodies in the form of small white specks about the size of a pin's head arose on the lower darkened side of the dung and a smaller number on the upper lighted side. However, *the upper lighted rudiments soon ceased to grow* and underwent abortion, whilst of the rudiments which had arisen in the lower darkened situations a few developed vigorously into mature fruit-bodies (Fig. 59, B). In the bottom-lighted Culture B, numerous rudiments of fruit-bodies arose on the upper darkened side of the dung and a smaller number on the lower lighted side. Here again the daylight showed itself to be the chief controlling factor in the selection of the rudiments, for *the lower, well-lighted rudiments soon ceased their development* and became aborted, whilst of the upper darkened rudiments a few eventually developed vigorously into mature fruit-bodies and shed an abundance of spores (Fig. 60). In both the cultures, as soon as the fruit-bodies had pushed upwards so as to touch the covering plates, more room for their development was provided by replacing the plates by bell-jars. In the Culture B, the stipes which were attached to the top of the dung at their bases only were so badly fixed in the substratum (Fig. 60, B) that it became necessary to prop them up in order to prevent the fruit-bodies from toppling over.

Cultures C and D. Set up simultaneously. After the dung had been inoculated with mycelium, the dish of C was placed in a dark-chamber from which all light was excluded and the dish of D was subjected to the maximum amount of daylight. In the Culture D, only three dung-balls were used and these were so placed that they did not quite touch one another. The crystallising dish which contained them, covered by its glass plate, was set on another glass plate which rested on an inverted iron tripod as in Culture B. The tripod, with the glass plate and dish above it, was then set at a window in a position as well lighted as possible. The dung-balls were lighted above, in front, and at the sides by direct

daylight, and below and behind by indirect daylight reflected in the required directions by a set of mirrors appropriately arranged.

In the entirely darkened Culture C, rudiments of fruit-bodies

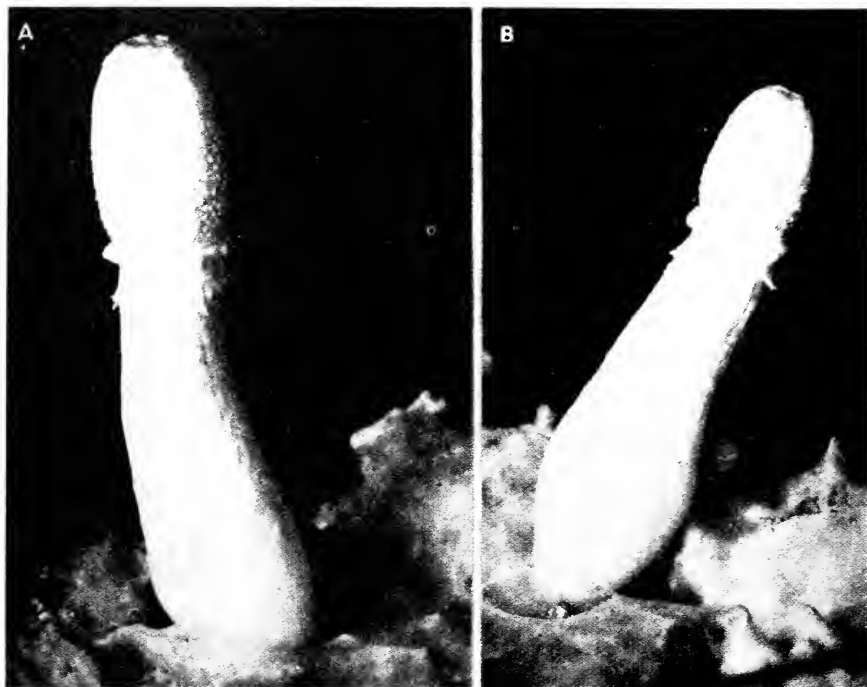


FIG. 60.—*Coprinus sterquilinus*. Fruit-bodies from Culture B, grown in a dish lighted from below and darkened above. Both the fruit-bodies A and B have developed from rudiments which came into existence on the top of the dung in the dark. No fruit-bodies developed from the lighted lower parts of the dung-balls. Each of the two fruit-bodies has a long solid stipe-base which passes gradually into the hollow stipe-shaft, and the two pilei, up to the present, are relatively retarded in development. Cf. Fig. 59 which shows fruit-bodies developed in top-lighted cultures. The two fruit-bodies A and B are both insecurely attached to the substratum. Later on they had to be supported with props to prevent them from falling on to their sides. Natural size.

arose in large numbers evenly all over the surface of the dung—at the bottom, top, and sides of each ball, wherever a free surface was exposed. In the end, a few rudiments *in all these situations* continued to develop and eventually produced fruit-bodies which shed spores; and the remaining rudiments all underwent abortion (Figs. 61 and 62). We thus find that, in the absence of light, the rudiments which are destined to develop into mature fruit-bodies do not of

necessity arise only on the under surfaces of the dung-balls, but arise just as freely upon the upper and lateral surfaces. Since the force of gravity acts equally well in darkness and in daylight, it is evident, by comparing the results of Culture C with those of Culture



FIG. 61.—*Coprinus sterquilinus*. Culture C, grown in complete darkness. Fruit-body rudiments can be seen on the top and sides of the horse dung. Two perfect fruit-bodies (in the foreground) are developing from rudiments which were at the bottom of the dung, and one perfect fruit-body (in the distance) from a rudiment which was at the top of the dung. Owing to the absence of light the solid stipe-bases have become very long and project above the surface of the horse dung, while the pilei which cap them have remained up to the present relatively small. Cf. Fig. 59, B, which shows a fruit-body of the same age grown in the light. Photographed 23 days after the culture was started from mycelium. Natural size.

A, that the low position of origin of the rudiments which develop into mature fruit-bodies in top-lighted cultures is decided not by the action of gravity but by that of light.

In the Culture D, where the maximum amount of daylight was brought to bear upon every part of the surface of each dung-ball, a number of rudiments of fruit-bodies appeared at the top, bottom, and sides of the balls; but they soon ceased to grow and became aborted. Ultimately, however, two rudiments which had evidently

been formed in dimly-lighted clefts of two of the balls continued their development and grew up into normal spore-producing fruit-bodies.

From the experiments just described it seems safe to conclude that daylight, whilst not able entirely to prevent the formation of fruit-body rudiments of *Coprinus sterquilinus* in the first place, inhibits the further growth of the rudiments at a very early stage of



FIG. 62.—*Coprinus sterquilinus*. Another aspect of Culture C, grown in complete darkness. Fruit-body rudiments can be seen on the top of the horse dung, and from three such rudiments three perfect fruit-bodies are developing. Here again, owing to the absence of light, the solid stipe-bases are well developed and project up above the surface of the dung, while the pilei which cap them have remained up to the present relatively small. Photographed 20 days after the culture was started from mycelium. Natural size.

their development. It must be pointed out, however, that *light has an inhibitory action only upon very small rudiments, i.e.* those having a diameter not exceeding about 1 mm. A rudiment which has originated in the dark and has attained a length of about 3 mm. is no longer checked in its further growth when exposed to light, but can develop into a normal fruit-body. In top-lighted cultures, such as Culture A, as we have seen, the upper lighted rudiments all become aborted, and the mature fruit-bodies all arise from rudiments which have come into existence on the darkened lower sides of the dung-balls. The successful rudiments, although arising in the dark, soon push their way upwards into the light; but, by the

time they have brought their upper ends into the light, they are too advanced to be influenced adversely by its action ; they therefore continue their further development unchecked. A rudiment which has succeeded in passing through what, so far as light is concerned, may be called its *critical stage of development* can afterwards withstand unharmed even strong sunlight. Should one find a full-grown fruit-body on a dung-mass in nature, one would be justified, therefore, in making the following reflection upon it. The fruit-body must have originated in a dark or dimly-lighted recess of the substratum, for otherwise the sun's light would have inhibited its development when it was a tiny rudiment. However, by the time it had grown sufficiently large to push upwards into the light, owing to an internal change in itself, it had become immune to the sun's inhibitory action. Hence, the later stages of its development took place freely in the light.

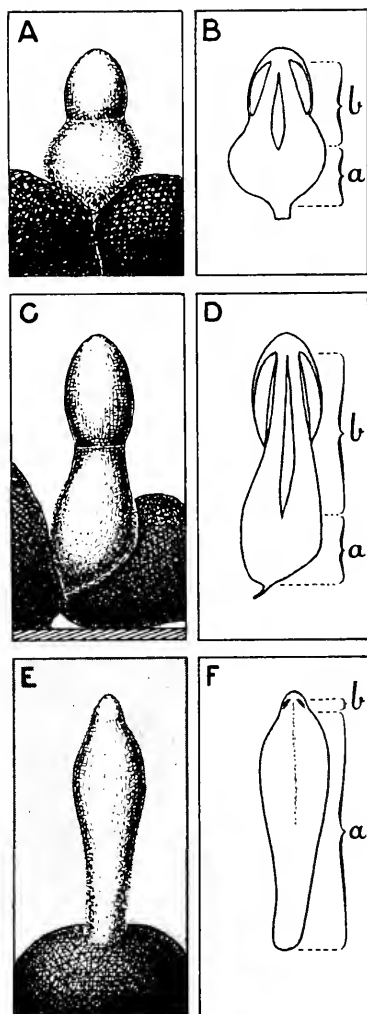


FIG. 63.—*Coprinus sterquilinus*. Comparison of two fruit-bodies grown in daylight with an equal-aged fruit-body grown in the dark ; to illustrate the fact that light inhibits the lengthening of the solid stipe-base. A and C, grown in the light ; E, grown in total darkness. B, D, and F, vertical sections of A, C, and E respectively ; a, the solid stipe-base ; b, the hollow stipe-shaft. Culture medium, sterilised horse-dung balls. The fruit-bodies A and C soon pushed up into the light, and therefore their stipe-base is relatively short and the pileus relatively advanced in development. The fruit-body E, owing to the absence of the inhibitory action of light, has continued to elongate its stipe-base, and its pileus is relatively very rudimentary. The pilei of the fruit-bodies A and C would expand about three days before the pileus of the fruit-body E. Natural size.

The ecological significance of the inhibitory action of light on tiny fruit-body rudiments will be pointed out in the Section on the mechanical fixation of the fruit-body.

The Morphogenic Effect of Light on Developing Fruit-bodies.—Light, in addition to exercising an inhibiting action upon the growth of tiny fruit-body rudiments, gives a morphogenic stimulus to young fruit-bodies, *i.e.* causes an alteration in their form. However, this moulding action of light is confined to the base of the stipe and does not affect the main shaft of the stipe or the pileus. In order to perceive the morphogenic effect of light it is necessary to compare the growth of two fruit-bodies in two cultures, one of which (like Culture A in the last Section) is top-lighted (Fig. 59, p. 106), and the other of which (like Culture C) has been kept in complete darkness (Figs. 61, 62, and 66 ; pp. 109, 110, and 116). In both light and darkness the pileus develops equally well and sheds spores in about equal abundance (for a mature fruit-body grown in complete darkness, *vide* Fig. 66) ; but there is a difference in the structure of the stipe. A fruit-body in a top-lighted culture, as we have seen, arises on the darkened lower surface of a dung-ball ; but, in response to the stimulus of gravity, it soon pushes its way upwards between the dung-balls. Its rudimentary pileus is thus soon brought into the light. As a result of the young fruit-body receiving light in this way, the base of the stipe—and by this is meant the *solid part of the stipe*, which will henceforth be called the *stipe-base*—becomes somewhat bulbous and, as a rule, does not attain a greater axial length than about 1 cm. (Figs. 63, B, and D ; 64, B ; and 65, A). On the other hand, a fruit-body which has been grown in complete darkness, wherever it may have arisen—at the top, bottom, or side of a dung-ball—has a solid stipe-base which, instead of being bulbous and only about 1 cm. in length, is always much elongated and 3–4 cm. in length (Figs. 63, F ; 64, D ; 65, B and C ; and 66). There is therefore a marked contrast between the form of a stipe-base of a fruit-body which has undergone most of its development in the light, and that of a fruit-body which has developed in complete darkness.

An elongated stipe-base of *Coprinus sterquilinus* is in reality a short and simple *pseudorhiza* of exactly the same morphological

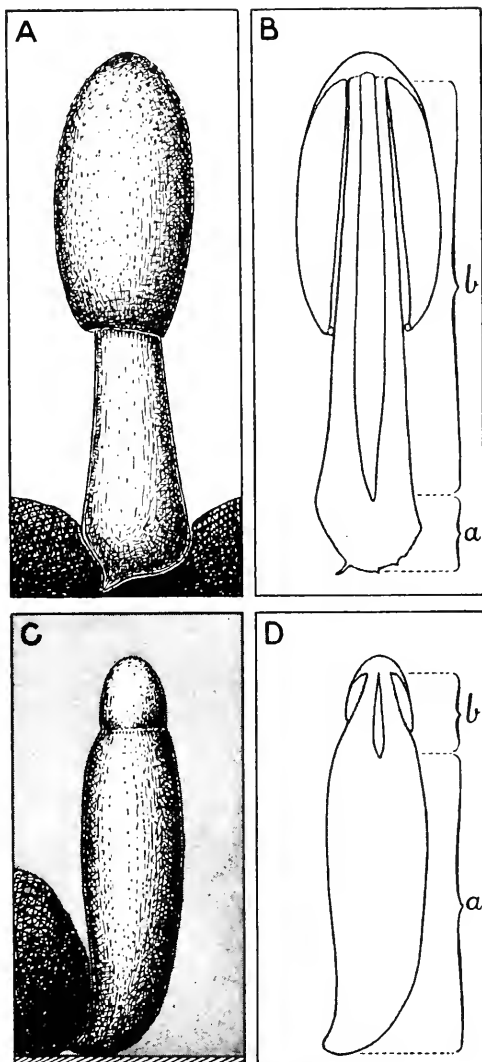
and physiological significance as the more highly developed and much longer pseudorhizae of *Collybia radicata* and *Coprinus macrorhizus* which will be described in a subsequent volume.

Fruit-bodies, whether grown in light or in darkness, complete the construction of their solid stipe-bases first. They then rapidly enlarge their pilei and subsequently elongate the main hollow shafts of their stipes. A hollow shaft of a stipe will henceforth be called a *stipe-shaft*.

Whilst the stipe-base of a fruit-body is developing, the pileus remains

FIG. 64.—*Coprinus sterquilinus*.

Comparison of a fruit-body grown in daylight with an equal-aged fruit-body grown in the dark; to illustrate the fact that light inhibits the lengthening of the solid stipe-base. A, grown in the light; C, grown in total darkness. B and D, vertical sections of A and C respectively; a, the solid stipe-base; b, the hollow stipe-shaft. Culture medium, sterilised horse-dung balls. The fruit-body A soon pushed up into the light with the result that light soon inhibited the growth in length of its stipe-base, which is therefore relatively short. The fruit-body C, having grown in the dark, was not able to push up into the light and therefore light has not inhibited the growth of its stipe-base which, in consequence, has grown to the maximum possible length. In A and B, the shortness of the stipe-base is correlated with an advanced state of development of the pileus; in C and D, the great length of the stipe-base is correlated with a relatively very rudimentary pileus. The pileus of A would have expanded and begun to shed spores about three days before that of C. Natural size.



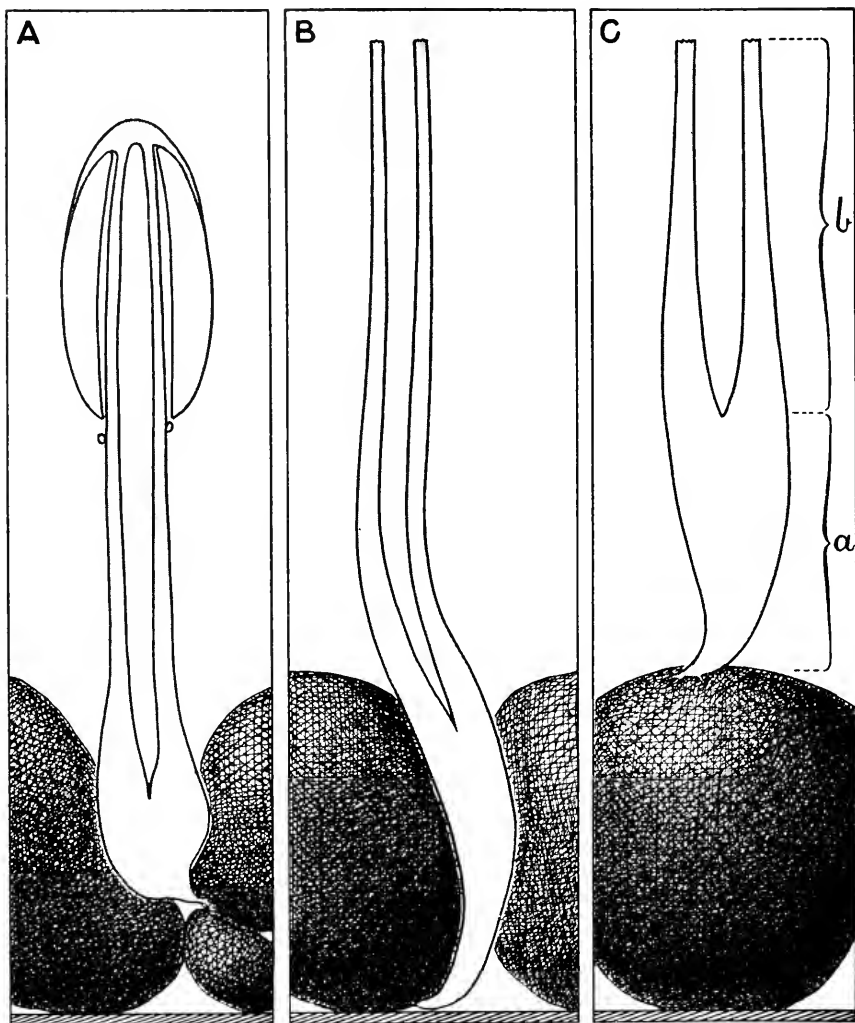


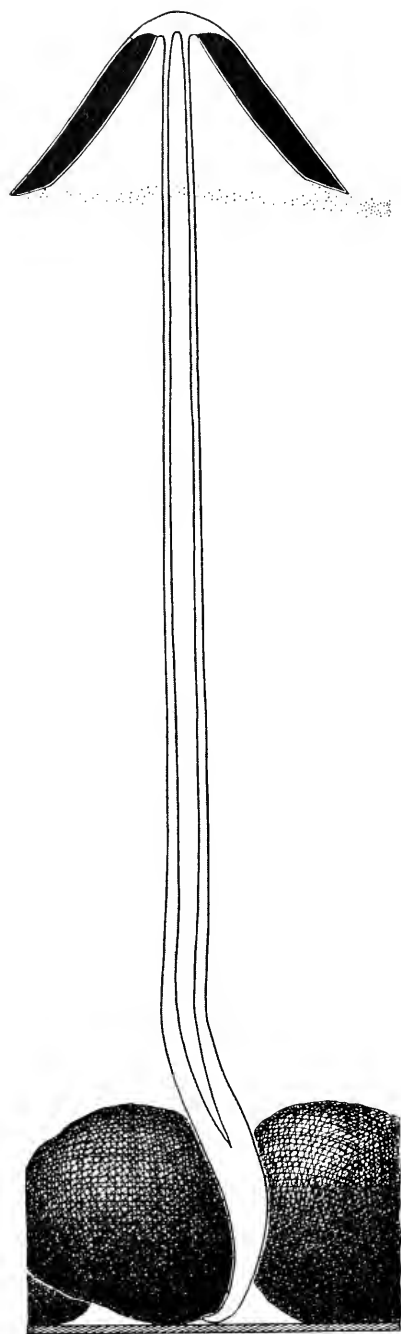
FIG. 65.—*Coprinus sterquilinus*. Comparison of the stipe-base of a fruit-body grown in daylight with the stipe-bases of two fruit-bodies grown in the dark. A, grown in the light; B and C, grown in total darkness (cf. Fig. 66); a, the solid stipe-base, b, the hollow stipe-shaft. Fruit-bodies shown in thin vertical section. Culture medium, sterilised horse-dung balls. The fruit-body A soon pushed its way up into the light and therefore light soon inhibited the growth in length of its solid stipe-base which, in consequence, is relatively short. The fruit-bodies B and C, having grown in the dark (cf. Figs. 61 and 62), could not push up into the light and therefore light has not inhibited the growth of the stipe-base which, in consequence, has grown to the maximum possible length. In A, light inhibited the development of fruit-body rudiments on the illuminated upper side of the dung-balls, but in C, in the absence of light, a fruit-body rudiment on the top of the dung-ball continued to develop. The fruit-body

very small (Figs. 63, F, and 64, D); but, as soon as the stipe-base has ceased to elongate, the pileus and contained stipe-shaft commence to grow rapidly. Now let us suppose that two fruit-bodies are grown in two cultures, one of which is top-lighted and the other completely darkened. The fruit-body in the light will soon complete the formation of its stipe-base, as this is relatively short; and it will then immediately proceed to the rapid enlargement of its pileus. On the other hand, the fruit-body grown in the dark will complete the formation of its stipe-base relatively slowly, for this, as a rule, is some four or five times longer than that of the fruit-body grown in the light (*cf.* in Fig. 63 B and D with F, and in Fig. 64 B with D). By actual observation it was found that, as a matter of fact, the development of the stipe-base of a darkened fruit-body takes two or three days longer than that of a top-lighted fruit-body. As soon as a darkened fruit-body has completed the development of its long stipe-base, it, too, immediately proceeds to the rapid enlargement of its pileus. Finally, the hollow shafts of the stipes and the pilei of both darkened and top-lighted fruit-bodies become equally well developed (*cf.* in Fig. 65 A with B and C; also *cf.* Fig. 65, A, with Fig. 66); but a comparison showed that a top-lighted fruit-body begins to shed its spores some two or three days before a darkened fruit-body. That a top-lighted fruit-body completes its whole development 2-3 days sooner than a darkened fruit-body appears to be solely due to the fact that a darkened fruit-body spends 2-3 days longer in the development of its stipe-base than a top-lighted fruit-body.

Although light completely inhibits the further growth of tiny rudiments exposed to its rays, yet, as we have just seen, light also hastens the later development of a fruit-body as a whole. This hastening, however, is simply due to the fact that *light inhibits the elongation of the stipe-base* and so permits the growth-energy of the fruit-body to be concentrated earlier than would otherwise be

FIG. 65—*cont.*

C differs from a fruit-body grown in the light (1) in developing on the upper side of a dung-ball and (2) in having a solid stipe-base protruding above the substratum. The fruit-body C, owing to the abnormal conditions of its development (absence of light), was insecurely fixed to its substratum, with the result that it toppled over and finally broke off at its base (*cf.* Fig. 60). Natural size.



the case upon the enlargement of the pileus and the elongation of the stipe-shaft.

Under natural conditions, the length of the solid stipe-base depends upon the depth within the faecal substratum at which the fruit-body rudiment has arisen. If the rudiment requires to push upwards for only 1 cm. to come into the light, its base will remain short and bulbous, 0·5–1 cm. long (Figs. 63, B and C, and 64, B). If, however, it has to push up 4 cm. or any greater distance to come into the light, its base will be 3–4 cm. long. If it has to push up a distance between 1 and 4 cm. in order to reach the light, its stipe-base will be of a length intermediate between 0·5 and 4 cm., corresponding to the amount of upward growth before the light is reached (Fig. 65, A). The general result of the morphogenic action of light on a fruit-body, therefore, is to regulate the length of the solid stipe-base in such a way

FIG. 66.—*Coprinus sterquilinus*. Vertical section through a mature fruit-body (part shown in Fig. 65, B) which developed in complete darkness. Culture medium, sterilised dung-balls. The fruit-body happened to originate on the bottom of a horse-dung ball. Its solid stipe-base, in the absence of light, has developed to the maximum length; the hollow stipe-shaft and the pileus are normal. The gills have just begun to discharge their spores. Reduced to two-thirds the natural size.

that the *stipe-base* is always situated in darkened crevices of the *substratum* and so that it never projects freely above the general upper surface of the *substratum*. The significance of this arrangement for the welfare of the fruit-body as a whole will be discussed in the Section treating of the mechanical fixation of the fruit-body.

The Relations of the Fruit-body with Gravity.—At its first origin, a fruit-body appears to be ageotropic and, for a short time afterwards, will grow outwards from its *substratum* in any direction—upwards, downwards, or sideways—without bending. However, as soon as the *stipe-base* has attained a length of a few millimetres and the *pileus* has become differentiated, the *stipe-base* gradually becomes sensitive to the stimulus of gravity and in consequence, during its further growth, bends slowly upwards so that it becomes curved. Finally, the *stipe* becomes very strongly negatively geotropic and it then pushes the *pileus* vertically upwards (Figs. 61, p. 109, and 66). The *pileus* does not respond to any geotropic stimulus: both its flesh and its gills are ageotropic.

The Upward Pressure of the Stipe.—It was noticed in the dish-cultures that, under the stimulus of gravity, a young *pileus* was often pushed upwards by the *stipe* with considerable force. This was made evident, sometimes by the raising of superincumbent dung-balls, and at other times by the actual breaking of a dung-ball into two or more pieces. It seemed of interest to measure the pressure exerted by the *stipe*, and the following experiment was therefore carried out.

A young fruit-body (Fig. 67), consisting of a well-developed basal bulb and a small *pileus*, was seated upon the bottom of a glass culture-dish in an upright position. The bulb was 1.4 cm. high and 0.7 cm. wide just below the *pileus*, and the *pileus* had attained a height of 1.4 cm. and a diameter of 0.8 cm. A piece of a collecting tube, 1.4 cm. high and 1.0 cm. internal diameter, with a flat end, was then inverted over the enlarging *pileus* so as to provide it with a cap; but, before this cap was placed in position, some cotton-wool was put in its interior so that, eventually, while the very top of the *pileus* would press against the glass end, the rounded upper sides of the *pileus* would press against the cotton-wool packing. After the cap had been adjusted, a brass weight of 20 grams was placed

upon its flattened end (Fig. 67). In the course of 5 hours this weight was raised by the upward pressure of the stipe a distance of 3 mm. Next day, the cap was found to be tightly fitted to the pileus owing to the enlargement of the latter. A thin test-tube was obtained and partly filled with shot, so that it weighed

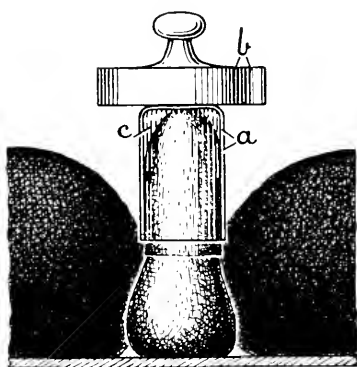


FIG. 67.—*Coprinus sterquilinus*. Measurement of the lifting power of a developing fruit-body. A young fruit-body, growing from a mycelium developed in sterilised horse-dung balls contained in a glass dish, has its base firmly seated on the bottom of the dish. A glass cap *a* has been put over the pileus and on this cap rests the brass weight *b* of 20 grams. The space at *c* was packed with cotton-wool (not here shown). The stipe continued to grow in length and, as it did so, it pushed the weight upwards. Natural size.

100 grams. This test-tube was then set in an upright position with its base resting upon the glass cap covering the pileus; and, to prevent its falling out of the vertical, it was enclosed by a loosely fitting glass cylinder (part of a larger test-tube) which was held in a clamp in the manner shown in Fig. 68. The weight of 100 grams was pushed upwards by the elongating stipe a distance of 4 mm. in 1 hour and 15 minutes. Additional shot was then added to the test-tube so that its weight was increased to 150 grams. This weight was then raised 1 mm. in 2 hours. As the test-tube was nearly filled with shot, instead of using more shot a further load of 50 grams was added by means of a brass weight which was placed on the top of the tube as shown in Fig. 68. The 200 grams were then

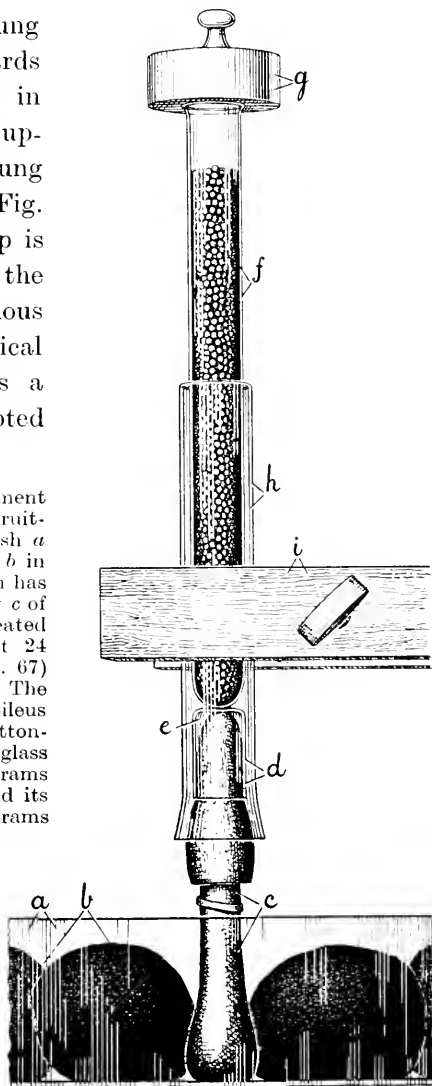
raised 2 mm. in 1 hour and 30 minutes. By adding another brass weight, the load was then increased to 300 grams; but this resulted in the stipe bending and breaking, so that the experiment came to an end.

The full load which was actually lifted by the fruit-body was 201.5 grams, for to the 200 grams represented by the test-tube with its shot and the brass weight above it must be added 1.5 grams, the weight of the glass cap covering the top of the pileus. Now 201.5 grams is approximately equal to 7 ounces or nearly half a

pound. Doubtless the stouter fruit-bodies of *Coprinus sterquilinus* are able with the tops of their stipes to exert an upward pressure of at least half a pound.

Small dung-balls weigh about 1 oz., large ones about 2 oz., and even very large less than 3 oz. The experiment just described, which shows that a fruit-body can exercise an upward push of at least 7 oz., makes it easy for us to understand how it is that a young fruit-body is able to push upwards two or three dung-balls which in dung-ball heaps may oppose its upward growth. The pileus of a young fruit-body is conical in form (Fig. 69, B), the solid flesh at its top is borne directly upon the end of the stipe, and its tip is mucilaginous and slippery. In its mechanical qualities it therefore resembles a root-cap and is admirably adapted

FIG. 68.—*Coprinus sterquilinus*. Measurement of the lifting power of a developing fruit-body. A large glass crystallising dish *a* contains sterilised horse-dung balls *b* in which has grown a mycelium which has given rise to the vertical fruit-body *c* of which the base of the stipe is firmly seated on the bottom of the dish. About 24 hours previously the pileus (cf. Fig. 67) was covered with the glass cap *d*. The space *e* between the sides of the pileus and the glass cap was filled with cotton-wool (not here shown). Upon the glass cap now rests a weight of 200 grams made up of (1) the test-tube *f* and its content of lead shot weighing 150 grams and (2) the brass weight *g* weighing 50 grams. The test-tube *f* and the weight *g* are kept in an approximately vertical position by the loose glass sheath *h* held by the wooden clamp *i*. The fruit-body pushed up the weight of 200 grams a distance of 2 mm. in an hour and a half. When the weight was increased to 300 grams, the stipe bent and broke. Reduced to two-thirds the natural size.



to be pushed upwards between obstacles, such as dung-balls, by the pressure exerted by the stipe. As was mentioned at the beginning of this Section, a dung-ball is sometimes broken into two or more pieces by the pileus, if it cannot be pushed to one side. A dung-ball has not sufficient mechanical resistance to withstand either the lifting or the splitting power of the young fruit-bodies. In nature, therefore, if a fruit-body arises in a dark crevice between dung-balls low down in a dung-ball heap, the fruit-body is able to exert sufficient pressure in its upward growth to enable it with certainty to escape from imprisonment and to reach the free surface of the substratum. Here again, therefore, we have another example of the beautiful manner in which *Coprinus sterquilinus* is adapted to its environment.

The stipe-shaft is a hollow cylinder. A transverse section was made through the stipe enclosed by the pileus in the experiment described above, and the area of the solid part was measured. This area was 0·045 square inches. The weight pushed up by the solid part of the stipe was the weight of the pileus plus the weights added artificially. Since the pileus weighed 2·5 grams, this weight was 204 grams in all. From this it was calculated that the pressure exerted by the solid part of the stipe in pushing up the pileus with its load had been approximately two-thirds of an atmosphere.¹

¹ There are a number of recorded instances in which a heavy stone has been raised by the expansive power of one or more agaricaceous fruit-bodies growing beneath it. Thus M. C. Cooke (*A Plain and Easy Account of British Fungi*, London, 1862, pp. 6-7) tells of "a large kitchen hearthstone which was forced up from its bed by an under-growing fungus and had to be relaid two or three times, until at last it reposed in peace, the old bed having been removed to a depth of six inches and a new foundation laid." Cooke also cites a comparable observation made by Dr. Carpenter: "Some years ago the town of Basingstoke was paved; and not many months afterwards the pavement was observed to exhibit an unevenness which could not readily be accounted for. In a short time after, the mystery was explained, for some of the heaviest stones were completely lifted out of their beds by the growth of large toadstools beneath them. One of the stones measured twenty-two inches by twenty-one, and weighed eighty-three pounds." Mr. C. V. B. Marquand of the Kew Herbarium has informed me that, when he was a boy, he observed at Guernsey, in a pavement, a large flagstone which had been raised on one side to a height of two or three inches. On looking beneath the stone, he observed there a group of hard, woody fruit-bodies (? *Lentinus lepidus*) which appeared to him to have been the causal agent in raising the stone. While it

The Heliotropic Effect of Light.—When the young fruit-bodies in one of the crystallising-dish cultures had grown up so as almost to touch the covering glass plate, the plate was removed and the open dish was set in a large chamber having glass sides, a glass top, and a zinc base (length, breadth, and height of the chamber respectively 42, 21, and 26 inches), the air of which was maintained in a sufficiently moist condition. The chamber was situated on a table about 10 feet from a window facing the open toward the west. Under these conditions the fruit-bodies expanded and shed their spores in a perfectly normal manner. As the fruit-bodies developed, they were subjected to unilateral window-light directed downwards to them at an angle of about 70° with the vertical.

In response to the unilateral illumination, each fruit-body, during its development, sloped its axis from the vertical slightly toward the source of light. The actual angles of slope for four fruit-bodies were 15° , 10° , 10° , and 8° respectively. Numerous observations of this kind made it evident that the stipes of *Coprinus sterquilinus* are slightly heliotropic when illuminated with unilateral light. A few experiments confirmed this deduction. In one of these, the dish containing a very young fruit-body, not quite one inch high, was turned round, so that the axis of the stipe was set at a slope of 16° from the vertical in a direction away from a window (Fig. 69, B). After 24 hours, owing to growth, the axis of the stipe had come to slope 8° toward the source of the light (Fig. 69, C), so that a heliotropic curvature of 24° had taken place. This slope toward the direction of the incident light-rays was maintained in the lower two-thirds of the stipe during the further elongation of the stipe and even during the expansion of the pileus (Fig. 69, D).

seems probable that the flagstone was actually raised by the fruit-bodies, as in the cases recorded by Cooke and Carpenter, there is just the possibility that the primary cause of the upheaval was a growing root upon which the fungus had vegetated, for it is well known that growing roots do actually disturb paving stones as they increase in thickness. There is no reason to doubt the general accuracy of the observations here cited. If a small and relatively fragile *Coprinus sterquilinus* fruit-body can exercise an upward pressure of about half a pound, several firm and large non-*Coprinus* fruit-bodies, like those of *Lentinus lepideus*, acting together, might well exert an upward pressure of many pounds and raise stones of considerable weight.

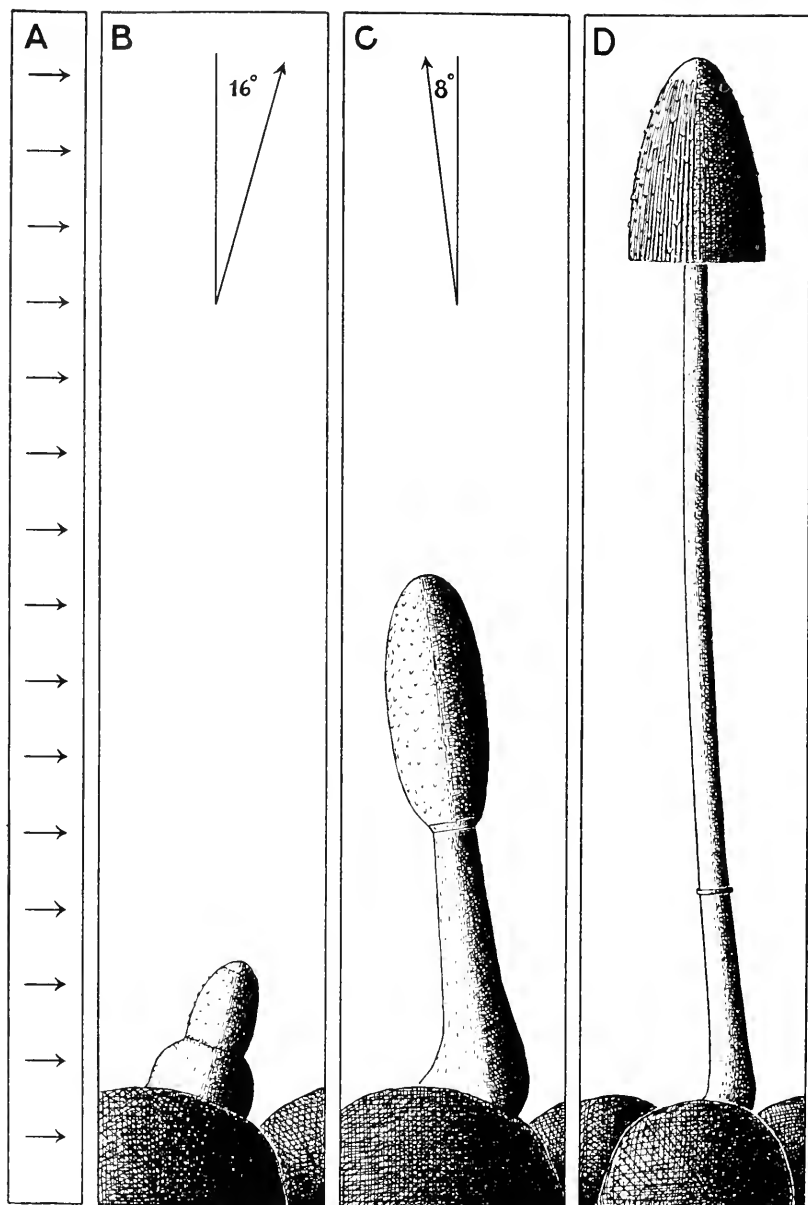


FIG. 69.—*Coprinus sterquilinus*. Heliotropic and geotropic reactions of a fruit-body exposed to unilateral illumination. Culture medium, sterilised horse-dung balls. A, the arrows indicate the general direction of the light to which the fruit-body was exposed during its development. B, a very young fruit-body, placed so that it is turned away from the light at an angle of 16° with the

A fruit-body which is very young—so that its pileus is only just becoming differentiated as a tiny cone on the top of the enlarging stipe-base, the whole being not more than about one quarter of an inch in length—appears to be not only ageotropic but also anheliotropic. Experiment showed that, at this stage of development, unilateral light, like gravity, is unable to bring about any tropic response. The rudiment of the fruit-body appears simply to grow outwards from the substratum in the direction of least mechanical resistance. As the young fruit-body becomes longer and longer, it becomes (as we have seen) responsive to the stimulus of gravity and so makes a geotropic curvature upwards through the substratum. As soon as the pileus emerges into the light, the stipe is found to have undergone a physiological change so that, in weak unilateral illumination, it gives a slight heliotropic response in the manner already described. It is not improbable that the young fruit-body becomes sensitive to the tropic stimulus of gravity sooner than to that of light.

The slope of a stipe at an angle of only about 10° from the vertical toward a window from which the light is striking the fruit-body at an angle of 70° from the vertical is evidently a resultant position due to the response of the stipe to two stimuli acting simultaneously in different directions. Gravity has tended to direct the stipe vertically upwards, while the light has tended to cause the stipe to grow away from the vertical at an angle of about 70° . It is evident that of the two stimuli gravity is by far the

FIG. 69—*cont.*

vertical. C, the same fruit-body 24 hours later than at B : it has grown in size and, in response to the stimuli of light and gravity, has bent toward the source of the light through an angle of 24° , so that it is now sloped toward the light at an angle of 8° with the vertical. This resultant position, taken up in response to the action of two stimuli—that of light tending to turn the fruit-body into a horizontal position and that of gravity tending to turn the fruit-body into a vertical position—indicates that the response of the stipe to the stimulus of gravity is greater than the response to the stimulus of light. D, the same fruit-body 24 hours later than at C : it has grown to a height of eleven inches and its pileus is expanding : the stipe, in its further growth after the stage shown at C, gradually ceased to be positively heliotropic and came to respond to the stimulus of gravity only, so that its upper end has become erect. Had the fruit-body responded as strongly and continuously to the stimulus of light as to the stimulus of gravity, it would have had a slope toward the light of 45° with the vertical, in which case, owing to its considerable weight and the distance of its centre of gravity from its base, it would have become insecurely fixed to its substratum and would have toppled over. B and C, natural size ; D, reduced to one-half the natural size.

stronger. This is highly advantageous to the fruit-body as a whole, as I shall now endeavour to show. One must bear in mind : (1) the general mechanical principle that the more upright a fruit-body, the less the moment of force acting about its base and tending to break the stipe or overturn the fruit-body as a whole ; and (2) the fact that the fruit-bodies of *Coprinus sterquilinus* are of considerable size and weight. The height of *C. sterquilinus* fruit-bodies is often 9–12 inches and the width of the fully expanded pilei 3–6 inches ; and, for one large fruit-body, the weight was found to be 27 grams and the centre of gravity 5·5 inches above the base of the stipe. Since the weight of the pileus is considerable and the stipe long, thin, and hollow, it is necessary for the mechanical stability of a fruit-body as a whole that the stipe shall be upright or almost upright. In open fields, where strong diffused light plays upon a dung-mass from all sides, the stipes usually grow up in an almost precisely vertical direction. If, however, a fruit-body grows upwards in such a position that one side of it is close to a dark obstacle (part of a dung-mass, etc.) and the other side looks toward a well-lighted open space, then the slight heliotropic reaction that takes place enables the stipe to slope very gently away from the obstacle, making with the vertical an angle of about 10° , with the result that, when the pileus comes to expand, the expansion is unhindered by the obstacle ; and this, in the end, is distinctly advantageous for the liberation of the spores. Since the slope from the vertical is only about 10° , the fruit-body can still maintain its fixed position without too much danger of falling. Were the angle of slope to be increased to say 45° or 60° , the moment of force acting about the base of the stipe would undoubtedly cause the fruit-body to fall down or break. In the laboratory this was often found to happen when, by slowly tilting the culture dish, the direction of the axis of the stipe of an expanded fruit-body was changed from the vertical or nearly vertical to the extent just indicated.

In Volume I of these *Researches*, I stated that the stipes of *Coprinus comatus* appear to be without response to the heliotropic stimulus of light.¹ Since it has now been shown that the stipes of *Coprinus sterquilinus* are slightly positively heliotropic, I suspect

¹ These *Researches*, vol. i, 1909, p. 76.

that the same is true for *Coprinus comatus*. Experiment alone can decide this matter and definitely prove whether I was in error or not. By experiment I was unable to detect any heliotropism for the fruit-bodies of *Psalliota campestris*, the Common Mushroom; but in this species the stipes are relatively short and stout. However, Miss Streeter, in a paper on the influence of gravity on the direction of growth of *Amanita*, has shown that the stipes of both *Amanita phalloides* Fr. and of *A. crenulata* Pk., which are relatively long and thin, are slightly heliotropic.¹ Indeed, her results for these two species exactly resemble my own for *Coprinus sterquilinus*, for she found that with unilateral illumination the stipes of the *Amanitae* become deflected toward the light so that they make an angle with the vertical of from 8° to 12°. From her observations and from my own upon *Coprinus sterquilinus* it seems highly probable that it will be found that a slight amount of positive heliotropism is characteristic of a great many, perhaps of the majority, of the larger Agaricineae. So far as the smaller Agaricineae are concerned, it will be remembered that, in the first volume of this work, in respect to small Coprini, e.g. *C. niveus* and *C. curtus* (there called *C. plicatiloides*), I pointed out: (1) that the stipe is at first ageotropic but strongly positively heliotropic, so that until it is half-grown in length its axis is maintained in a direction *coinciding with the direction of the incident rays of light*; and (2) that, just before the expansion of the pileus, and only then, the stipe undergoes a remarkable change in its physiological state; it becomes strongly negatively geotropic and anheliotropic.² For smaller fruit-bodies, like those of the smaller Coprini, this kind of reaction is without any serious mechanical disadvantages, for the weight of the fruit-bodies is small and the centre of gravity not far away from the base of the stipe, in consequence of which there is no danger of the curved stipes becoming broken or displaced by the weight of themselves and their pilei.³ If the stipes of the relatively gigantic fruit-bodies

¹ S. G. Streeter, "The Influence of Gravity on the Direction of Growth of *Amanita*," *Botanical Gazette*, vol. 48, 1909, p. 415.

² These *Researches*, vol. i, pp. 67-69.

³ The ecological advantage in the successive reactions of the stipes to light and gravity is that these reactions enable small fruit-bodies to emerge safely from between the dung-balls. *Ibid.*

of *Coprinus sterquilinus* were to make the same bold curvatures in response to light as those made by the tiny fruit-bodies of *C. niveus* and *C. curtus*, there is no doubt that disaster would result: the stipes would either topple down or break. We may conclude, therefore, that smaller Agarics and larger ones have different modes of reacting to the tropic stimuli of light and gravity, each mode being beautifully suited to the mechanical qualities (size and weight) of the two classes of fruit-bodies.

The Mechanical Fixation of the Fruit-body.—In order that the millions of spores which are developed on the under side of the pileus shall be freely liberated into the air and thus be carried off by the wind, it is necessary that the fruit-body as a whole shall be firmly fixed in its substratum. Mechanical fixation is as important for a fruit-body as it is for a tree. In a Phanerogam the fixed position of a stem is fraught with various advantages: it enables the leaves to take up an optimum position in respect to the light and so aids the process of photosynthesis, it allows of the flowers being oriented so that pollination may be properly effected, and it often permits of the seeds or fruits being liberated in such a manner that they may be readily disseminated by the wind or by animals. In such a fungus as *Coprinus sterquilinus*, however, the fixed position of the fruit-body is related to one function only, the production and liberation of the spores; but upon the successful carrying out of this the whole life of the species—its persistence from one generation to the next—absolutely depends.

The fruit-bodies of *Coprinus sterquilinus*, which require to be firmly fixed, are, as we have seen, of considerable size and weight. In a normal, well-grown fruit-body, the height is 6–10 inches and the width of the fully-expanded pileus 2–4 inches; but, in very large fruit-bodies which appear from time to time in artificial horse-dung cultures, the height may be as great as 11–12 inches and the width of the pileus 5–6 inches. One large fruit-body, as already recorded on a previous page, was found to have a weight of 27 grams, or nearly 1 oz., and to have its centre of gravity situated at a distance of 5·5 inches from the base of the stipe. *Coprinus sterquilinus* is strictly coprophilous and always grows on horse dung. Therefore, the substratum to be considered in connection

with the problem of fixation in nature consists of masses of irregularly piled dung-balls as dropped in pastures and other places by grazing horses.

The various *external* factors which play a part in the fixation of a fruit-body are as follows :

(1) Light which inhibits the development of the tiny rudiments of fruit-bodies which may arise on the illuminated surfaces of the substratum.

(2) Light which inhibits the growth in length of the solid stipe-base as soon as the pileus has been pushed up into the light.

(3) Gravity which causes the stipes of young fruit-bodies to push upwards through the substratum.

(4) The structure of the dung-mass which consists of more or less spherical or oval lumps of considerable size piled together so that there are crevices between them.

The *internal* factors which, independently of external stimuli, play a part in the fixation of a fruit-body are as follows :

(1) The continued development of a fruit-body rudiment in the dark, if its growth is not inhibited by the growth of a more vigorous neighbouring rudiment.

(2) The early development of a long, solid, stout stipe-base in the dark, and the correlated delayed development of the combined stipe-shaft and pileus beyond the size of a small cone.

(3) The dense coat of free hyphae which grow outwards in all directions from the exterior of the stipe-case and the lowest part of the stipe-shaft.

We shall now attempt to correlate the data which have been brought forward in this and in the preceding Sections, and by this means describe what may be termed the *act of fixation* of a fruit-body.

Tiny rudiments of fruit-bodies, not larger than about the size of a pin's head, may and do arise simultaneously at many points everywhere over the surface of the infected substratum, *i.e.* in the light, in the dark, on upper or lower surfaces of the dung-balls or in crevices between the dung-balls. Of the scores or hundreds of rudiments thus brought into existence only one or a few can possibly survive, owing to the size of the fruit-bodies to be produced and

the limitation in the quantity of nutrient substances stored up within the mycelium of the dung-balls. Certain rudiments are therefore selected for further development. The selection takes place in two stages.

The first selection of fruit-body rudiments is carried out as follows. All the illuminated rudiments on the upper exposed surfaces of the substratum are prevented from growing beyond the size of a pin's head by the inhibitory action of light. The result of this is that, under natural conditions, *no mature fruit-body can ever come into existence with the base of its stipe seated directly upon the top or exposed upper sides of a dung-ball*. Such a position, as experiments with cultures kept entirely in the dark have shown, would be so insecure that the fruit-bodies as they grew in length would topple over by their own weight and be ruined physiologically. The inhibitory action of the light on the tiny rudiments, therefore, prevents fruit-bodies from ever taking up the insecure positions indicated.

The rudiments remaining after the first selection are those which have arisen on darkened surfaces, *i.e.* in darkened crevices on the under sides of the dung-balls. At first, these rudiments are numerous ; but, by means of a second selection, only one or a very few of them are permitted to undergo further development. This second selection depends on nutrition and mechanical opportunity. If the mass of dung which is infected with the mycelium is large, then several fruit-bodies—each one as a rule distantly situated from its nearest neighbours—may come to maturity ; but, if the infected substratum is limited to one or a very few dung-balls, then only one fruit-body may be ultimately produced. Those rudiments which happen to occupy the most favourable situations in respect to the mycelium and to free space for growth obtain the most nutriment, grow fastest, and are those which are destined to survive and attain maturity. The more vigorous development of one rudiment causes the entire inhibition of growth of all the smaller neighbouring rudiments within a certain radius. This inhibition of growth appears to be due to starvation : a favoured rudiment robs its less fortunate companions of the food substances which would otherwise fall to their share. The contents of the mycelium

always flow to the fruit-body which grows fastest, and feebler rudiments have no power to divert the stream when once it has begun to move quickly in one direction. The mycelium in a mass of dung-balls just before fruit-body rudiments are formed upon it consists of a single three-dimensional closely-knit network; for all the mycelia which have been produced from many separate spores, or all the hyphae of a mycelium produced from a single spore, have by this time become linked up through the formation of vast numbers of bridging hyphae. A single developing favoured rudiment, so far as the contents of this mycelial network is concerned, has a definite sphere of influence which extends radially for not more than a few inches. Within this sphere of influence the rudiment extracts ultimately all the nutrient contents of the hyphae, and with these it builds up its stipe-base, its stipe-shaft, and its pileus. The final stage of activity of the emptying hyphae, thus drawn upon, is probably the absorption of water for conduction to the stipe when this is undergoing its extremely rapid elongation. When the mycelium is very extensive, there is room for the development of several favoured rudiments at separate points in the substratum; and then each rudiment has its own particular sphere of influence. In the end, under normal conditions, the spheres of influence divide up the mycelium completely, so that the whole of its contents are employed in fruit-body production.

A favoured rudiment which is able to continue its development after the second and final selection process is, therefore, one which *has arisen at some little distance*, an inch or so, *below the upper illuminated surface of the substratum*, in a darkened crevice under a dung-ball or between two or more dung-balls. Such a rudiment now becomes differentiated into a solid stipe-base crowned with a relatively tiny conical pileus enclosing the rudiment of the hollow stipe-shaft. The developmental energy then becomes concentrated upon the stipe-base. This swells and, as it grows in length, pushes the tiny conical pileus away from the surface of the substratum in the direction of least mechanical resistance. This is possible owing to the fact that, at this stage of development, the rudiment is ageotropic and the stipe-base readily permits its form to be moulded

in response to the resistance offered by mechanical obstacles. One can observe the moulding effect of the pressure exercised by dung-balls and glass by looking at developing rudiments through the glass base of culture dishes. The developing rudiment is also anheliotropic ; but, since at this stage of its existence it is growing in a dark crevice, even if it were positively heliotropic its direction of growth could not as yet be influenced by light. The solid stipe-base, as it continues to grow in length, gradually becomes sensitive to the stimulus of gravity. As a consequence, it makes a geotropic curvature upwards and so brings the axis of the tiny pileus into a vertical position. The stipe-base is thick and very solid, and the pileus with which it is crowned is very small, pointed, hard, and smooth. The whole of the young fruit-body, for such we may now call it, forms an admirable boring instrument for penetrating through the superincumbent faecal dung-mass. The top of the solid stipe-base, as it elongates, pushes the pileus upwards with so great a pressure that the latter is compelled to force its way between two or three dung-balls or even to break a way upwards through the actual substance of the dung. Since, as we have seen, a young fruit-body is able to lift a mass of nearly half a pound in weight and individual dung-balls only weigh from one to three ounces each, this forcing of a way upwards into the light is accomplished quite easily by the pressure of the growing stipe-base. Until the pileus has been pushed up into the light, its development is delayed : it remains as a small cone capping the upper end of the cylindrical stipe-base. The significance of this delayed development is obvious : it allows of the pileus being used as an efficient point for the boring instrument, *i.e.* for the fruit-body as a whole, during the time when the penetration of the dung-mass must be accomplished.

As soon as the pileus has emerged above the dung-mass into the light, the light acts upon the fruit-body in such a way that the further elongation of the solid stipe-base is inhibited. The length of the stipe-base is therefore decided by the distance through which the young fruit-body has to grow upwards through the dung-mass in order to bring the pileus into the light. The advantages accruing from the fruit-body having a solid base to its stipe where the solid

base is immersed in the substratum are of a mechanical nature, and shortly they will be considered in detail. There would be no special advantage in the solid stipe-base being continued upwards into the air above the surface of the substratum; and the inhibitory action of the light on the growth of the stipe-base always prevents projecting stipe-bases from coming into existence. In this matter the sun's light is a beautiful regulator.

With the emergence of the pileus into the light and the inhibition of the growth in length of the solid stipe-base, the growth-energy of the fruit-body becomes concentrated upon the development of the small conical pileus, which now quickly assumes a barrel-shaped form and becomes many times larger. After a few days, the barrel-shaped pileus attains its full growth in size and the spores begin to develop at the surface of the gills. The stipe-shaft, which is hollow, then grows rapidly in length and raises the pileus to a height of from 6 to 10 inches from the ground. If for any reason, when the stipe-shaft is growing upwards in response to the directing stimulus of gravity, one side of the fruit-body is darkened owing to the existence there of some dark object, the stipe becomes inclined away from the object toward the source of greatest illumination and may thus become deflected from the vertical by an angle of about 10° . The pileus, whilst still being raised to its final altitude in space, expands umbrella-wise. As soon as it has become campanulate, the process of autodigestion sets in and the spores begin to be discharged. Finally, the pileus becomes flattened so as to form a disc with more or less revolute edges. The gills and the peripheral portions of the pileus are gradually destroyed by autodigestion and, by the end of the spore-discharge period which continues for about 8 hours, the pileus has become reduced to a mere tattered stump. A few hours after spore-discharge has ceased, the shaft of the stipe loses its vitality and collapses. The most persistent part of the whole fruit-body is that which comes into existence first, namely, the stipe-base; but this, too, dies a few hours after the collapse of the stipe-shaft.

In consequence of the fruit-body developing in the manner which has just been described, the full-grown stipe is found to consist of two distinct parts which are differently constructed and

are differently located : a short *stipe-base* which is solid and fixed within the faecal substratum, and a long *stipe-shaft* which is hollow and projects upwards in the air. The mechanical functions of the stipe-base are : (1) to push up the young pileus into the light, (2) to hold the whole fruit-body firmly fixed in one position during the development of the stipe-shaft and pileus, and (3) to support the weight of the stipe-shaft and pileus even when these are full-grown. On the other hand, the stipe-shaft has but one mechanical function—that of supporting the pileus. The differences in the structure of the stipe-base and of the stipe-shaft are correlated with the different functions which these organs carry out. Each organ has to withstand a particular set of mechanical stresses and strains. The solidity of the stipe-base is advantageous because the stipe-base is subjected to : (1) lateral compression from opposing parts of the substratum in crevices when the stipe-base is undergoing its initial thickening ; (2) longitudinal compression from the weight and resistance of the substratum during the raising of the pileus upwards into the light ; (3) longitudinal compression, subsequently, from the weight of the full-grown stipe-shaft and pileus ; (4) lateral compression or crushing strains arising from the dung-balls which the stipe-base during its growth in length has forced somewhat apart ; and, finally, (5) irregular strains, due to the weight of the stipe-shaft and pileus and the action of the wind upon these organs, which have a tendency to force the stipe-base laterally out of its original position. Evidently, the mechanical demands made upon the stipe-base owing to the various strains and stresses just enumerated, which tend to crush it either longitudinally or laterally, are very considerable ; and it seems, therefore, only in accordance with the fitness of things that the stipe-base should be a solid and not a hollow cylinder. The length of the stipe-base just suffices to allow this organ to come to the upper lighted surface of the substratum. It is within the substratum that the greatest mechanical strains and stresses operate ; and it is precisely within the substratum that the stipe-base is situated. The stipe-shaft is hollow and long. Since it is aurally situated, it does not require to withstand lateral crushing strains like those to which the stipe-base is subjected, but only to support the weight of the pileus and

to resist bending. Under these conditions, as every engineer would allow, its hollow cylindrical form is the most efficient possible ; it has the same significance as the hollow cylindrical form of grass-stems and of the upright iron columns used for supporting buildings. If the stipe-shaft, with the same height and amount of material, were to consist of a narrower solid cylinder, its ability to hold the pileus in a fixed position in space during the discharge of the spores would be seriously impaired. The great length of the stipe-shaft is of considerable biological advantage in that it permits the stipe-shaft to raise the pileus high into the air, and thus to place the gills in a position where they may liberate their spores so that these may be carried off by the wind without interference from neighbouring obstacles.

Conclusion.—From the foregoing description of the mode of development of a fruit-body of *Coprinus sterquilinus* it is clear that the stipe becomes fixed in its faecal substratum only owing to a combination of all the external and internal factors for the fixation process which were summarised at the beginning of the previous Section. These factors may here be briefly considered again. Light inhibits the development of all the tiny rudiments of fruit-bodies which happen to arise on the illuminated surface of the substratum, so that only rudiments which develop in dark recesses beneath or between dung-balls can possibly produce mature fruit-bodies. In the dark a rudiment may continue its development if this is not inhibited by the growth of a more vigorous neighbouring rudiment. A favoured rudiment growing in the dark at a short distance below the free surface of the substratum devotes its growth-energy to the development of a solid, stout stipe-base, while the pileus which caps it remains small and conical, its development being delayed. A negative geotropic stimulus soon causes the stipe-base to push the rudimentary pileus upwards between the dung-balls or through the dung-mass into the light ; and, as soon as the pileus has reached the light and with this the free upper surface of the substratum, the light inhibits the further growth in length of the solid stipe-base ; and then the pileus and hollow aerial stipe-shaft are developed to their full extent in succession. The structure of the dung-mass, which consists of more or less spherical

or oval lumps of considerable size piled together, is also an obvious factor in fixation ; for it provides the crevice or recess in which a



FIG. 70.—*Panaeolus campanulatus*. A fruit-body which came up in a mass of horse dung in the laboratory. It arose between two dung-balls in a dark crevice. Probably any fruit-body rudiments that appeared on the upper sides of the dung-balls were inhibited from further growth by the action of light. Owing to its low place of origin, the stipe was well fixed mechanically between the two dung-balls. Horse-dung balls collected at Haslemere, England. Photographed in the laboratory at Winnipeg. Natural size.

favoured rudiment may begin its development and then, by the mutual opposition of its parts, presses against the sides of the stipe-base so that the latter becomes firmly wedged in between the dung-balls, between or through which it has grown upwards. The very base or first-formed part of the stipe-base is attached to the mycelium within a dung-ball and to the mycelial stratum, with its mycelial strands, which clothes the exterior of the dung-ball. The shaft of the stipe-base, which may be from 0·5 to 4 cm. long, according to the distance upwards through which the stipe-base grows to bring the rudimentary pileus into the light, is held laterally

by the before-mentioned mutual pressure of opposing dung-balls, etc. Thus, the whole stipe-base becomes fixed within the substratum in a most effective manner. Its hold upon the dung is doubtless rendered all the more complete by the densely packed

hyphae which grow more or less radially outwards from all over the surface of the stipe-base and also from the lowest part of the stipe-shaft. These hyphae, which give a woolly or peronate appearance to the parts which they clothe, make their way into crevices between particles of the dung and to some extent contribute to the stability of the fruit-body as a whole. With the elucidation of the various factors which play a part in the fixation of a fruit-body, the problem of fixation for *Coprinus sterquilinus* may be considered to have been solved.

In other large or fairly large agarics which grow on horse dung, both in the field and in the laboratory one may observe that the base of the stipe of a fruit-body is never attached to the exposed top of a dung-ball but always at its side or near its base. A basal or low lateral attachment of a fruit-body to a dung-ball is well shown for *Panaeolus campanulatus* in Fig. 70 and for *Galera tenera* in Fig. 71, and similar illustrations for *Stropharia semiglobata* and *Anellaria separata* are given in Volume II, Figs. 114 and 123 (pp. 328 and 348) respectively. It is probable that in these fungi, as in *Coprinus sterquilinus*, light inhibits the development of all fruit-body rudiments which are exposed to its rays, and thus determines that those rudiments which are destined to



FIG. 71.—*Galera tenera*. A fruit-body which came up on a mass of horse dung in the laboratory. It arose beneath a dung-ball in a dark place. Probably, if other fruit-body rudiments appeared on the upper side of the dung-ball, they were inhibited from further growth by the action of light. Owing to its low place of origin, the stipe was well fixed mechanically between the dung-ball and the base of the glass dish. Horse-dung ball collected at Haslemere, England. Photographed in the laboratory at Winnipeg. Natural size.

continue their development shall begin their growth in a position favourable for the mechanical fixation of the mature fruit-bodies, *i.e.* in a dark crevice in the substratum. It is also probable that the other factors concerned in the fixation of the fruit-bodies of *Panaeolus campanulatus*, *Galera tenera*, *Stropharia semiglobata*, *Anellaria separata*, and large horse-dung agarics in general are essentially the same as those which have been found to be concerned in the fixation of the fruit-bodies of *Coprinus sterquilinus*.

PART II

SOCIAL ORGANISATION AND SEX IN THE HYMENOMYCETES

CHAPTER I

SOCIAL ORGANISATION IN *COPRINUS STERQUILINUS* AND OTHER FUNGI

Social Organisation in Animals and Plants—The Algae—The Myxobacteriaceae—The Acrasieae—The Mycetozoa—The Phycomycetes—The Hymenomycetes—Hyphal Fusions and Clamp-connexions in the Hymenomycetes—Social Organisation in *Coprinus sterquilinus*—Social Organisation in Other Hymenomycetes—Social Organisation in Other Fungi—The Various Functions of Hyphal Fusions in the Hymenomycetes—Sex and Hyphal Fusions in the Hymenomycetes

Social Organisation in Animals and Plants.—Social organisation is known to exist in a greater or less degree in many animals. It has attained a high state of perfection in certain insects, *e.g.* the hive-bee, ants, and termites, while in man it has been an essential factor in the progress of civilisation.

In the Bryophyta, the Pteridophyta, and the Phanerogamia, the individuals of one and the same species, when they come into contact on the same substratum, always compete with one another; and, in these great plant phyla, there is no species known in which neighbouring individuals become united in such a way that some only of the individuals produce spores or seeds while others remain sterile, the former reproducing the species at the expense of the latter. Where in certain trees actual fusion takes place between the roots or stems of adjacent individuals which happen to come into contact (Figs. 72 and 73), the fusions appear to be of but little physiological importance and they do not lead to any division of labour between the individuals concerned.

In some of the Thallophyta, namely, certain Algae, the Myxobacteriaceae, the Acrasieae, the Mycetozoa, and certain Fungi, the individuals of one and the same species become associated so as to form remarkable social communities.

The Algae.—In the Algae, various degrees of co-operation between individuals of the same species are exhibited. Among the Cyanophyceae, the Chlorophyceae, and the Phaeophyceae there are numerous species in which many individual plants live together in the same mass of jelly on an equal basis (Figs. 74 and 75). The communal organisation in these colonies is of the lowest possible



FIG. 72.—Two large Douglas Fir trees (*Pseudotsuga taxifolia*) united together by the natural graftage of lateral roots. The united roots, which protrude above the forest floor, are well seen in the centre of the photograph. Photograph by C. A. Pemberton, taken in British Columbia, Canada.

type. A slight advance on this (Fig. 76) is to be found in such branched colonial forms as *Dinobryon* (Phaeophyceae), *Gomphonema* (Chrysophyceae), and *Licmophora* (Bacillariales). In *Hydrodictyon*, the Water-net, a large number of zoospores arrange themselves to form a non-motile coenobium of coenocytes, a curiously reticulated structure in which all the coenocytes have equal value so far as reproduction is concerned (Figs. 77 and 78).

In the Volvocaceae, as is well known, there is a series of algae—usually regarded as colonial—whose highest forms, in the structure and functions of the cells of which they are composed, clearly

display a morphological differentiation of structure and a physiological division of labour. In this connection, it need only be mentioned that in one species¹ of *Volvox*—the culminating genus in

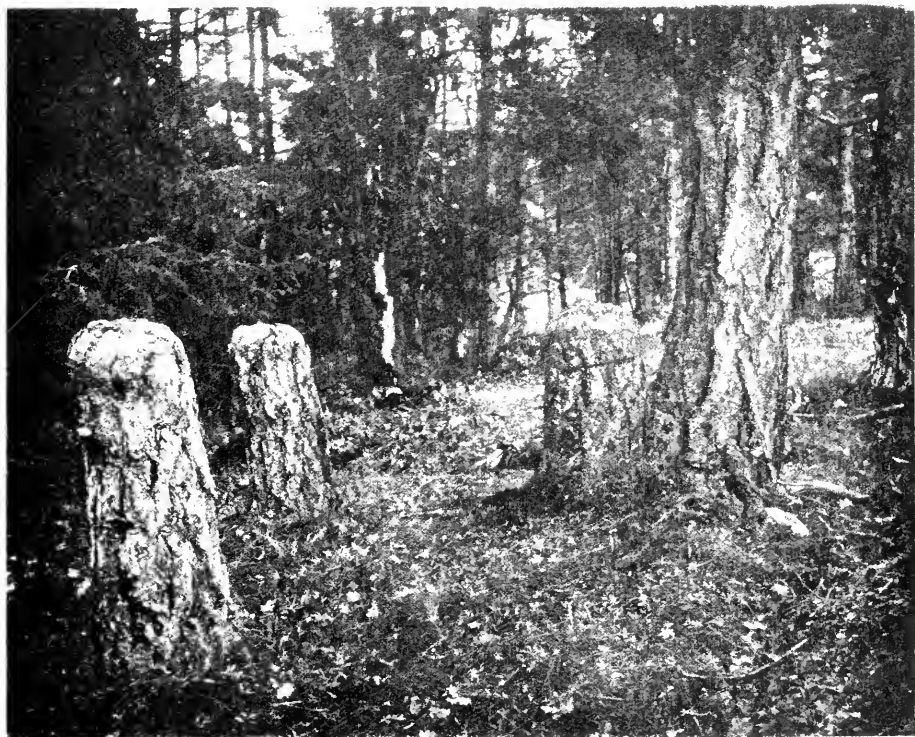


FIG. 73.—Three stumps of Douglas Fir trees which, in the course of many years, have become overgrown and completely healed over. They are now "living posts." The supply of food materials to the stumps was made possible owing to their roots being naturally grafted to undamaged Douglas Fir trees, one of which is shown in the foreground on the right. Photograph by C. A. Pemberton, taken in British Columbia, Canada.

the evolution of motile coenobia—there may be as many as 22,000 cells, the vast majority of which are purely vegetative in function, only a few ever taking part in either sexual or asexual reproduction

¹ *Volvox globator*. In an asexual individual there are 1500–16,400 cells, and in a sexual individual 10,000–22,000. *Vide* L. Klein, "Morphologische und biologische Studien über die Gattung *Volvox*," *Jahrb. f. wiss. Bot.*, Bd. XX, 1889, p. 146.

(cf. Fig. 79). Notwithstanding that algologists in general¹ hold the view that a Volvox is a colony of cells, the writer, with Janet,² is inclined to regard a Volvox as a highly organised, multicellular *individual plant*. In support of this view it may be said: (1) that the cells of which a Volvox is composed are all derived by cell-division from one and the same mother-cell; (2) that the units of

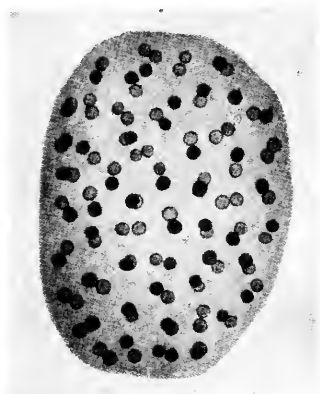


FIG. 74.—*Aphanocapsa Grevillei*, a simple colonial Blue-green Alga. The cells are globose and aggregated to form a small colony within a common homogeneous gelatinous integument. Found in Yorkshire, England, and drawn by G. S. West. From his *British Freshwater Algae* (1904). Magnification, 450.

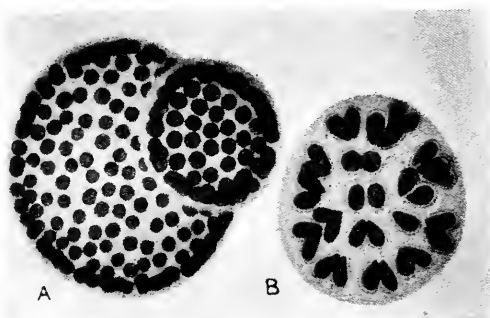


FIG. 75.—Colonial Blue-green Algae, in which the individual cells are held together in a mass of mucus. A, *Coelosphaerium Kützingianum* from Loch Neagh in Ireland: the cells are arranged just within the periphery of a spherical mass of mucus. B, *Gomphosphaeria aponina*, from Keston Common, Kent, England: the cells are fewer and more scattered, pyriform in shape, arranged in pairs, and are disposed chiefly toward the periphery of the mass of mucus. Found and drawn by G. S. West. From his *British Freshwater Algae* (1904). Magnification, 350.

the cell-mass never live an independent life but are in contact with one another from the first; and (3) that the cell-mass, from an early stage of development, is united into one whole by means of

¹ E.g. F. Oltmanns, *Morphologie und Biologie der Algen*, Jena, 1923, p. 56. F. E. Fritsch in his revision of G. S. West's *British Fresh-water Algae* (Cambridge, 1927, p. 26) says, in respect to Volvox, "such colonial forms are scarcely to be distinguished from multicellular individuals," but he does not tell us how Volvox differs from a multicellular individual.

² C. Janet, *Le Volvox*, Limoges, 1912, pp. 12, 16, 17. In treating of the phylogeny of Volvox, Janet says that, at one stage in evolution: "D'individus, les Flagellates sont devenus des plastids constitutifs d'une association définitive, et la colonie est devenue un individu."

well-developed protoplasmic connections (plasmodesmae).¹ The development of a Volvox sphere, as Janet² has pointed out, is comparable with the development of the embryo of animals up to the blastula stage; and, just as a blastula is considered as an individual organism, so also, it seems to the writer, should be an adult Volvox.

The Myxobacteriaceae.—In *Chondromyces crocatus* B. and C., one of the Myxobacteriaceae, the individual bacteria (Fig. 80) by mass action build up an aerial gelatinous branched stalk or cystophore, somewhat less than 1 mm. high, upon the ends of which the living rod-like bacteria are packed together in little oval cysts (Fig. 81, no. 6, and Fig. 80, C and D). These cysts have definite walls, are grouped together in globose heads and, at first sight, appear to resemble the spores of a mould fungus.³ According to Thaxter,⁴ during the formation of a cystophore (Fig. 81, nos. 1–6), a “mass

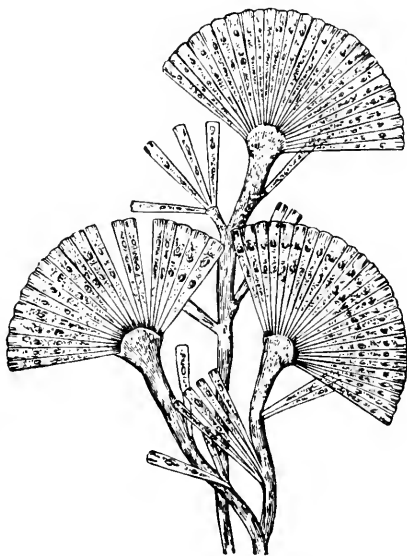


FIG. 76.—*Licmophora flabellata*, a marine colonial Diatom. The wedge-shaped frustules are coherent so as to form a flabellum or fan at the end of each branch of a gelatinous stalk which is attached to a sea-weed. From Carpenter's *The Microscope and its Revelations* (London, ed. IV, 1868, p. 291).

¹ The text-book illustrations always show the plasmodesmae in Volvox but not in Eudorina. Eudorina is usually represented as having its cells widely separated from one another by thick masses of jelly; but, even in this organism, protoplasmic strands stretch between the cells although they can be seen only after staining (Fritsch, *loc. cit.*, p. 76).

² C. Janet, *loc. cit.*

³ *Chondromyces crocatus* has orange-red cystophores and straw-yellow cysts. I have seen it several times in the laboratory at Winnipeg, where it has appeared on old, rather wet, horse-dung cultures. It first attracted my attention in these cultures as it grew on the rotting stipes of *Galera bulbifera*, one of the coprophilous Hymenomycetes. A pure culture made by sowing cysts obtained from a dung culture provided the material from which Fig. 80 was drawn.

⁴ R. Thaxter, “On the Myxobacteriaceae, a new order of Schizomycetes,” *Botanical Gazette*, vol. xvii, 1892, p. 396.

of rods moving upwards on one another continually leaves behind and below it an external layer at its base which has become slightly hardened by exposure to the air and is composed partly of the gelatinous matrix and partly of individuals which soon become indistinguishable in it." After a time, under suitable conditions, the cysts are able to "germinate" (Fig. 80, D): the bacterial contents of each cyst leave the cyst wall as an empty shell, the escaped individual rods then divide rapidly, and thus is initiated a new period of vegetative activity. Social organisation in *Chondromyces crocatus* is therefore exhibited in two ways:

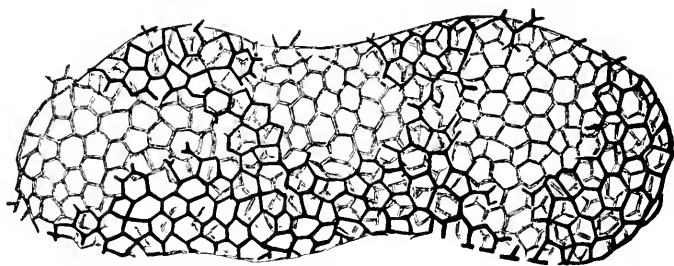


FIG. 77.—*Hydrodictyon utriculatum*, known as the Water-net, a green fresh-water plankton alga. The net, shown natural size, is composed of cylindrical cells, each of which was once a swarming biciliate zoospore. The net is therefore of colonial origin. After Cohn, from Bennett and Murray's *Handbook of Cryptogamic Botany* (1889).

(1) in that, in the building up of the fructification, to use the words of Thaxter,¹ "there is a concerted action of aggregates of individuals toward a definite end, namely, the production of a more or less highly differentiated resting state"; and (2) in that the bacteria which happen to be enclosed in a stalk during its formation are sacrificed for the welfare of the bacteria enclosed within the cysts, only the latter serving to reproduce the species.

The Acrasieae.—In *Dictyostelium mucoroides* (Fig. 82, A and B), one of the Acrasieae, social organisation is even more advanced than in the Myxobacteriaceae. In this species, as was first observed

¹ R. Thaxter, "On the Myxobacteriaceae, a new order of Schizomycetes," *Botanical Gazette*, vol. xvii, 1892, p. 392.

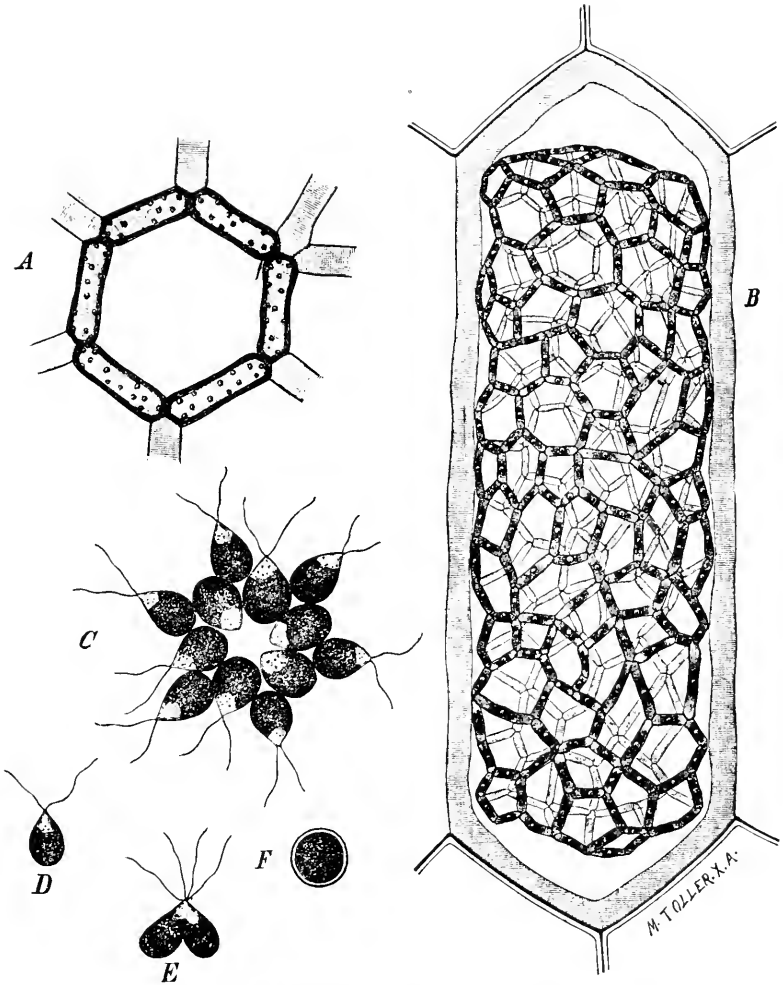


FIG. 78.—*Hydrodictyon utriculatum*, a Water-net. Drawings which illustrate the origin of a net from a combination of zoospores. A, a piece of a net showing the cylindrical cells of which it is composed. B, a cylindrical cell with a swollen cell-wall, containing a net which has just been formed from a combination of zoospores. C, a number of zoospores, which were produced in a cylindrical cell like B, connected together by fine protoplasmic threads (threads not shown) and each provided with two cilia (cilia of the three inner zoospores directed toward centre not shown); the zoospores are moving about, but keeping the arrangement shown, and soon they would each develop into a cylindrical cell and so form a net like that in B. D, a single gamete. E, two gametes becoming fused together; F, a zygote. From G. Klebs, *Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen*, Jena, 1896, Fig. 4, p. 134. Magnification: A and B, 120; C–F, 1000.

by van Tieghem¹ and subsequently confirmed by Brefeld,² the myxamoebae, derived from spores, at first wander about and feed and multiply themselves by fission separately (Fig. 82, E and F).

Then all the units come together and form an aggregate plasmodium in which the individual myxamoebae retain their individuality (Fig. 82, G, H, and I). Finally, the aggregate plasmodium builds up a long, sterile, cellular stalk crowned by a globular mass of spores (Fig. 82, J). The stalk-forming myxamoebae, on becoming transformed into stalk-cells, are thereby immobilised and rendered incapable of reproducing the species; nevertheless, they contribute to the welfare of the other myxamoebae — those which eventually form the spores — by constructing an apparatus

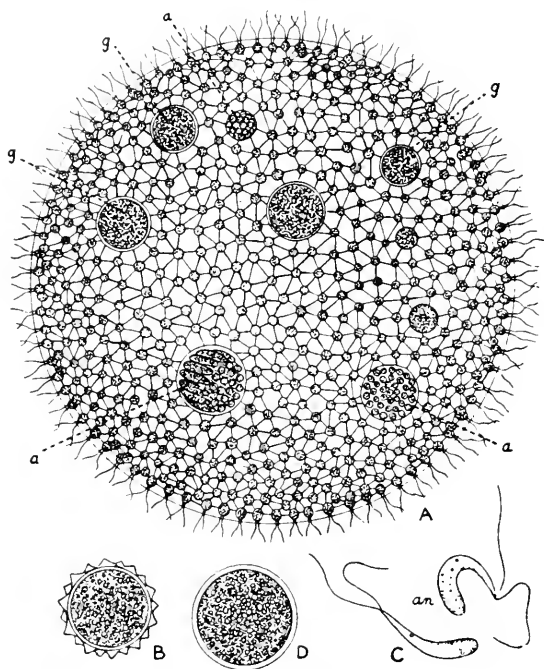


FIG. 79.—A, C, and D, *Volvox aureus*. Described by West and others as a colony of cells, but regarded by the author as an individual plant. A, a "monoecious sexual colony" (West), from Rawcliffe Common, Yorkshire, England: *a*, androgonidia; *g*, gynogonidia; the other cells all vegetative and united by protoplasmic processes. C, two antherozoids. D, a ripe oospore. B, a ripe oospore of *V. globator*. From G. S. West's *British Freshwater Algae* (1904). Magnification: A, 210; B and D, 475.

up which the latter may ascend from the substratum to take up a position which presumably is favourable for spore-dispersion.³

¹ Ph. van Tieghem, "Sur quelques Myxomycètes à plasmode agrégé," *Bull. Soc. Bot. France*, T. XXVII, 1880, p. 317.

² O. Brefeld, *Untersuchungen über Pilze*, Leipzig, Heft VI, 1884.

³ *Dictyostelium mucoroides* often appears on old horse-dung cultures at Winnipeg. The communal activities of the myxamoebae illustrated in Fig. 82 were observed in artificial cultures.

The Mycetozoa.—In the Mycetozoa, a number of young plasmodia of the same species, each derived from a zygote formed by the fusion of two amoebulae, may fuse together into a single compound plasmodium.¹ When such a compound plasmodium is formed, the cytoplasmic masses of the component plasmodia become

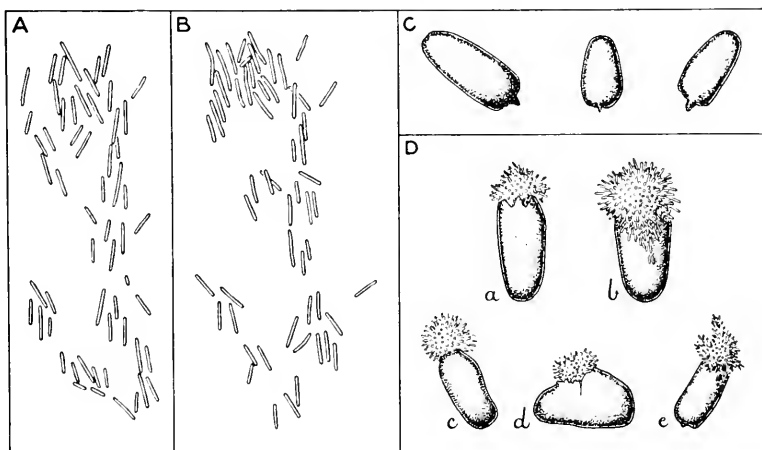


FIG. 80.—*Chondromyces crocatus*, one of the Myxobacteriaceae. A, a troupe of bacteria in a hanging drop of cleared dung-agar, drawn with the *camera-lucida*. B, the same troupe two minutes later: all of the bacteria have changed their positions but have kept together as though they have influenced one another's movements. C, three cysts which have been removed from a fruit-body (cf. Fig. 81, no. 6). D, cysts germinating in dung-agar: *a*, a cyst from which bacteria are emerging; *b*, the same cyst four hours later; *c* and *e*, two other cysts which have opened apically; *d*, a cyst which has opened laterally. Winnipeg material, found on rotting stipes of small Coprini in a horse-dung culture. Drawn by A. H. R. Buller and Ruth Macrae. Magnification: A and B, about 1066; C and D, 402.

indistinguishably united, while the various nuclei brought together and embedded in it remain separate from one another, each retaining its identity. A compound plasmodium of the kind just described may grow in size with an increase in the number of its nuclei, and eventually it may become transformed into fruit-bodies. Thus, a number of small, simple plasmodia, by combining with one another, may pool their resources in respect to both their vegetative functions and the production of sporangia and spores. The formation of a compound plasmodium from a number of simple ones is another

¹ G. Lister, *A Monograph of the Mycetozoa*, third edition, London, 1925, p. xviii.



example of social organisation, and it is doubtless advantageous to the species in that often for competition between numerous small and weak plasmodia, each of which might be too small to fruit, there is substituted co-operation which leads to the formation of a vigorous plasmodium large enough to produce one or more fruit-bodies of the size characteristic for the species.

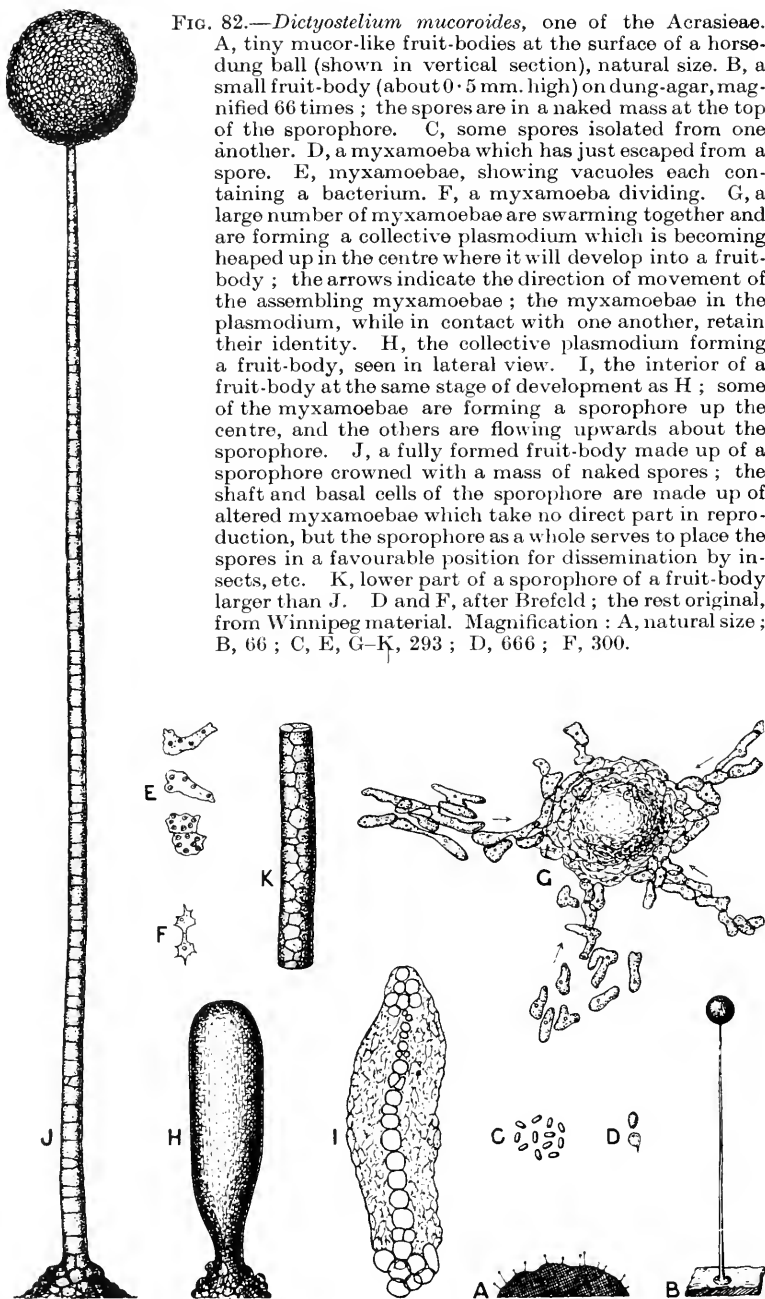
It has been observed by Jahn¹ that, in the Mycetozoa, *e.g.* *Didymium squamulosum*, a zygote, after beginning to feed as a plasmodium, is able to ingest an unpaired amoebula into a large digestive vacuole and there dissolve and absorb it. This cannibalism, while causing the extermination of useless amoebulae, provides a source of food for zygotes which, after feeding in the usual way on bacteria, etc., may grow into large fruiting plasmodia. Since it has to do with the action of one member of a species on another with some advantage resulting to the species as a whole, it may perhaps be regarded as a social act. The destruction of useless drones in a bee-hive by the worker bees after the marriage of the queen is somewhat analogous from the social point of view, for it, too, leads to a reduction in the number of associated individuals with an ensuing advantage in respect to the food supply.

In the fruit-bodies of *Trichia decipiens*, *Hemitrichia abietina*, *H. clavata*, etc., and most species of *Arcyria*, *e.g.* *Arcyria pomiformis* (Fig. 83), certain spores, namely those in the sporangium, become disseminated by the wind and have a reproductive function,

FIG. 81.—*Chondromyces crocatus*, one of the Myxobacteriaceae, which exhibits social organisation in the formation of its fruiting structures. Nos. 1–6, successive conditions of cyst formation shown by as many individual colonies. No. 1 : *a*, a mass of rods just rising from the substratum and becoming constricted at the base ; *b*, a smaller mass which has begun to secrete a cystophore and has become two-lobed preparatory to branching. No. 2, a more advanced mass, preparing to produce three branches. Nos. 3 and 4, nearly mature cystophores showing branching of the third and fourth order, the ultimate masses beginning in some instances (*a a*) to bud out into cysts. No. 5, a specimen cultivated on moist agar, the cystophore unusually stout, the ultimate masses almost wholly converted into immature cysts. No. 6, a specimen grown on straw showing normal habit ; the cysts are not yet mature. From a drawing kindly lent by Roland Thaxter ; the same as Plate XXI in Vol. XVII of the *Botanical Gazette*. The cystophores have an average height of 0·6 mm. and rarely attain 1 mm.

¹ *Vide* G. Lister, *loc. cit.* Her Fig. 3 which shows zygotes containing ingested amoebulae was drawn from stained preparations lent by Dr. Jahn.

FIG. 82.—*Dictyostelium mucoroides*, one of the Acrasieae. A, tiny mucor-like fruit-bodies at the surface of a horse-dung ball (shown in vertical section), natural size. B, a small fruit-body (about 0.5 mm. high) on dung-agar, magnified 66 times; the spores are in a naked mass at the top of the sporophore. C, some spores isolated from one another. D, a myxamoeba which has just escaped from a spore. E, myxamoebae, showing vacuoles each containing a bacterium. F, a myxamoeba dividing. G, a large number of myxamoebae are swarming together and are forming a collective plasmodium which is becoming heaped up in the centre where it will develop into a fruit-body; the arrows indicate the direction of movement of the assembling myxamoebae; the myxamoebae in the plasmodium, while in contact with one another, retain their identity. H, the collective plasmodium forming a fruit-body, seen in lateral view. I, the interior of a fruit-body at the same stage of development as H; some of the myxamoebae are forming a sporophore up the centre, and the others are flowing upwards about the sporophore. J, a fully formed fruit-body made up of a sporophore crowned with a mass of naked spores; the shaft and basal cells of the sporophore are made up of altered myxamoebae which take no direct part in reproduction, but the sporophore as a whole serves to place the spores in a favourable position for dissemination by insects, etc. K, lower part of a sporophore of a fruit-body larger than J. D and F, after Brefeld; the rest original, from Winnipeg material. Magnification: A, natural size; B, 66; C, E, G–K, 293; D, 666; F, 300.



while other "spores," called *spore-like cells*,¹ which fill up the central cavity of the stalk of the fruit-body and make it solid (Fig. 83, B), are never dispersed and take no part in reproduction. Although the spore-like cells do not themselves reproduce the

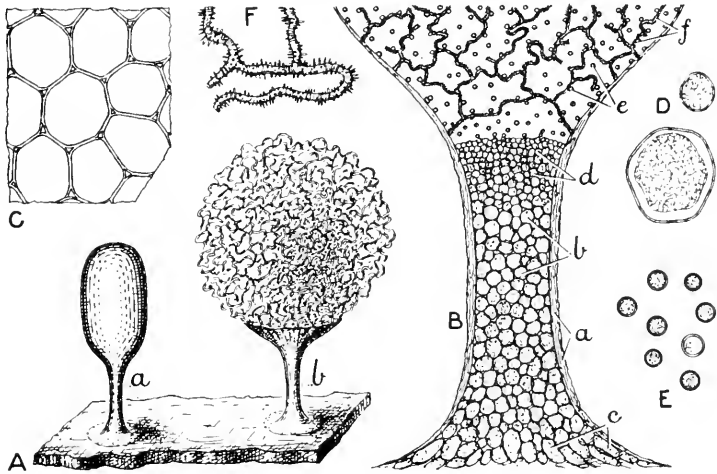


FIG. 83.—Sporangia of *Arcyria pomiformis*. To show the *spore-like cells* which fill up and strengthen the stalk, but are never set free and take no direct part in reproduction. A, two fruit-bodies on a piece of wood : *a*, mature but unopened ; *b*, the sporangium has dehisced and the capillitium is exposed to view. B, a vertical section through the cup and stalk of a sporangium which has dehisced : *a*, the membranous stalk-wall ; *b*, the spore-like cells cohering into a mass of cells which completely fills the stalk ; *c*, larger, more irregular spore-like cells near the hypothallus ; *d*, smaller spore-like cells at the top of the stalk, above which in the cup are the true spores *e* lying free within the network of the capillitium *f*. C, a group of spore-like cells of medium size, in transverse section, to show their hexagonal form, their walls, and the air-spaces between them (cell-contents omitted). D, two spore-like cells, one very large, the other small ; the cell-contents of the large one are contracted. E, a group of spores. F, a piece of the capillitium. Drawn from material and a preparation supplied by Miss Gulielma Lister. Magnification : A, about 20 ; B, about 84 ; C-F, about 333.

species, nevertheless they are of advantage to the species in that they strengthen the apparatus in which the ordinary spores are developed. If we are to regard the spore-like cells as morphologically equivalent to the ordinary spores, we must admit that, in the Mycetozoa under discussion, we have another instance of social

¹ For species containing these spore-like cells *vide* Miss Lister's *Monograph*. Miss Lister kindly supplied me with material and a preparation from which my Fig. 83 was drawn.

organisation in which some individuals of a species (the spore-like cells) are sacrificed for the good of others (the ordinary spores) which may develop further and keep the species in existence.

The Phycomycetes.—In the lower Fungi there are many species, e.g. *Mucor mucedo* (Fig. 84), *Pilobolus longipes*, and *Saprolegnia ferax*, in which, apart from the requirements of sex, the individual mycelia never unite but live to themselves alone, no social organisation being displayed.¹

The Hymenomycetes.—In the Hymenomycetes—the leading group of the Higher Fungi—non-sexual hyphal fusions between hyphae of the same mycelium, or between hyphae of two or more mycelia of the same species, have long been known and can readily be observed in hanging-drop cultures. Often, in larger cultures inoculated with many spores of a single species, the mycelia all unite to form a large compound mycelium, and then this complex netted thallus acts as a unit in the formation of one or more large fruit-bodies. This social organisation, hitherto, has not received the attention which it deserves; but it must frequently come into play in Hymenomycetes living under natural conditions and be of considerable importance in aiding the survival of all those species which exhibit it.

Hyphal Fusions and Clamp-connexions in the Hymenomycetes.—In preparation for a more complete discussion of social organisation in the Hymenomycetes, a few words must here be said about the origin and nature of their hyphal fusions and clamp-connexions.

The union of two separate hyphae with one another in the Hymenomycetes takes place in two ways, with results which have already been illustrated for the mycelium of *Panus stypticus* in Volume III.

(1) In the older part of a mycelium a branch hypha may develop laterally from another hypha in the usual manner. Then, owing to apical growth, its end may happen to come near the side of

¹ It must not be supposed, however, that hyphal fusions between vegetative hyphae are entirely unknown in the Phycomycetes, for they occur in *Syncephalis*. For illustrations *vide* W. Zopf, "Zur Kenntniss der Infektionskrankheiten niederer Thiere und Pflanzen," No. IV, *Nova Acta Acad. Caes. L.-C. Nat. Cur.*, Bd. LII, 1888, Taf. 6, Figs. 4, 5. Like Zopf, I have observed hyphal fusions in the mycelium of a *Syncephalis* parasitic on *Pilobolus*.

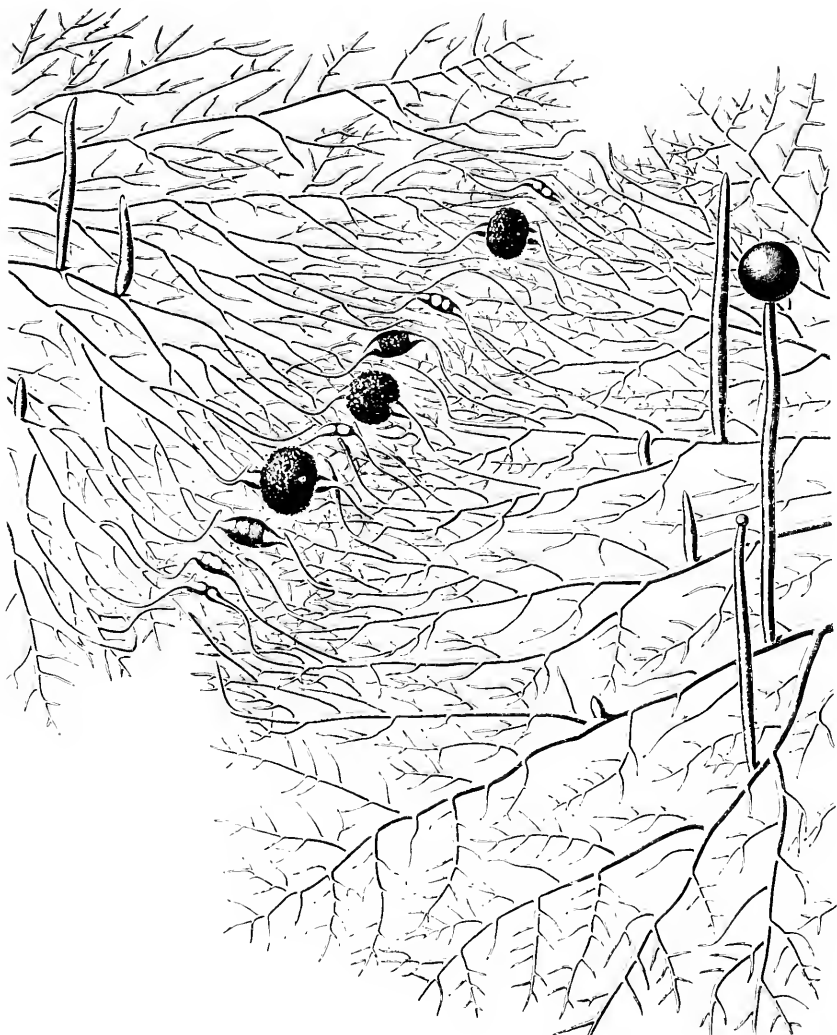


FIG. 84.—*Mucor mucedo*, one of the heterothallic Mucorineae. Two monosporous mycelia of opposite sex, one belonging to a (+) sexual strain and the other to a (-) sexual strain, meeting and forming a line of zygospores along the area of contact. To show the absence of hyphal fusions in either of the two mycelia, and also the absence of hyphal fusions between the two mycelia except where such fusions are necessary for the union of gametes and the consequent production of zygospores. From the lower left passing upwards obliquely to the right may be seen: young zygothores approaching, meeting, and forming progametes; the formation of walls separating the gametes from the suspensors; the disappearance of the central wall separating the two gametes; and the mature, rough-coated zygospore which has been formed as a result of the fusion of the gametes. After A. F. Blakeslee. Copied in black-and-white by the author from the *Tabulae Botanicae* published by Gebrüder Borntraeger in Berlin. Highly magnified.

another hypha of considerable length. The branch hypha, as if in response to a chemotropic stimulus, then grows directly toward the other hypha and meets it at a greater or smaller angle (often at right angles). The apex of one of the two hyphae thus comes to be pressed against the lateral wall of the other hypha. The cell-walls separating the two hyphae from one another are then dissolved, presumably by an enzyme, and fusion is complete: the two hyphae are now united by their cell-walls and there is an open passage between them. This type of hyphal fusion is illustrated for the mycelium of *Panus stypticus* at *h* and *m* in Fig. 176, D, in Volume III (p. 414) and again in this chapter for most of the hyphal fusions shown for *Coprinus sterquilinus* in Figs. 87, 88, and 89, and for *C. lagopus* in Fig. 96.

(2) Two hyphae in the course of their development have crossed so that at one place they are very near to one another but not in contact. At the place which marks the shortest distance between the two crossed hyphae, one of the hyphae, on the side nearest to the other hypha, sends out a lateral branch which grows directly toward the other hypha and fuses with it. Such a special branch hypha, since it comes to form a bridge between two other hyphae, may be called a *bridging hypha*. Bridging hyphae are illustrated for the mycelium of *Panus stypticus* at *c* in B and C and at *d* in D, Fig. 176, Volume III (p. 414) and again in this chapter for *Coprinus lagopus* at *f* and *g* in Fig. 96 (p. 170). Two bridging hyphae in the mycelium of a Pyrenomycete, *Pleurage anserina*, are well shown in Fig. 102, B (p. 178).

It seems possible that the growth of one hypha toward another, which it meets unerringly and with which it subsequently fuses, is due to a chemotropic stimulus provided by some substance excreted by the non-growing hypha; but this has not been proved, and it may be that the phenomenon has an entirely different explanation. In this connexion an experimental enquiry is desirable.

In the life-history of the Hymenomycetes in general there are two kinds of mycelia: the *haploid* and the *diploid*. A haploid mycelium comes into existence when a spore germinates. It is characterised by having isolated nuclei—often only one in each cell—and simple cross-walls. A haploid mycelium is transformed

into a diploid one spontaneously in a homothallic species, and after union with another mycelium containing complementary sexual factors in a heterothallic species. A diploid mycelium is characterised by having its nuclei in pairs, by the pairs of nuclei dividing conjugately, and by the formation of a clamp-connexion at each septum. The stages in the formation of a clamp-connexion in a diploid mycelium, as represented by Mlle Bensaude, are shown in Fig. 85.

Social Organisation in *Coprinus sterquilinus*.—

To illustrate the phenomenon of social organisation in the Hymenomycetes it will be convenient to consider the mode of growth and reproduction in *Coprinus sterquilinus*. This fungus has already been treated of from other points of view in Volume III¹ and in the three preceding chapters of this Volume IV. Its mycelium is homothallic and grows on horse dung, while its fruit-bodies are of large

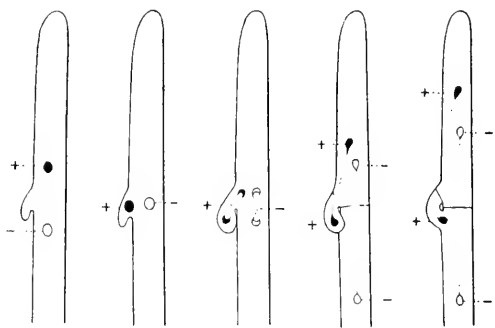


FIG. 85.—Diagram showing, from left to right, a conjugate nuclear division and the formation of a clamp-connexion in a hypha of a diploid mycelium of *Coprinus lagopus* (= *C. fimetarius* of Mlle Bensaude). The (+) sign indicates a nucleus of one sex, and the (−) sign a nucleus of opposite sex. The (+) nucleus and the (−) nucleus have been derived respectively from a (+) nucleus and a (−) nucleus which became associated with one another when a (+) mycelium and a (−) mycelium fused with one another and diploidised one another. In stage no. 1, a hook is growing backwards; in stage no. 2 the (+) nucleus has passed into the hook and the (−) nucleus is by the side of it in the main hypha; in stage no. 3, the (+) and the (−) nuclei are dividing conjugately; in stage no. 4, two septa are being formed one across the main hypha and one across the top of the hook, and for the moment the (+) nucleus is a prisoner in the hook-cell; in stage no. 5, the lower end of the hook has fused with the main hypha and the (+) nucleus is passing downwards toward its fellow (−) nucleus. Each of the two new cells now contains a pair of conjugate nuclei and the clamp-connexion has been completely formed. Copied by Dr. Nellie Carter from Mlle Bensaude's *Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes* (Nemours, 1918).

size. A single average fruit-body is so large that, during its development and expansion, it drains and exhausts a large mass of mycelium. In the production of a large fruit-body (Fig. 86), the mycelium used up may be that present either in two or more small

¹ These *Researches*, vol. iii, 1924, pp. 177–259.

dung-balls, or in one large dung-ball, or in part of a very large dung-ball. For our purpose, in what follows, it will be assumed that *a*



FIG. 86.—*Coprinus sterquilinus*. A large fruit-body coming up in a pure horse-dung culture. The pileus is expanding and has just begun to discharge its spores. The fruit-body, in the course of its development, drew upon and exhausted the mycelium in more than one dung-ball. Photographed at Winnipeg. Natural size.

single fruit-body completely uses up the mycelium in a single dung-ball.

The development of the mycelium of *Coprinus sterquilinus* in a hanging drop of dung-agar is illustrated diagrammatically in

Figs. 87 and 88. In Fig. 87, in the centre of the drop, is a single spore which has germinated and has given rise to a mycelium the branches of which tend to grow outwards in a radial direction. The mycelium was at first completely haploid, *i.e.* its nuclei were separated and non-conjugate (*cf.* Fig. 52, p. 83), and in this condition its cross-walls were all simple and not provided with clamp-connexions (*cf.* Fig. 51, F, p. 81); but now, the mycelium is turning from the haploid to the diploid condition, *i.e.* the nuclei are becoming paired and are beginning to divide conjugately. This change is always accompanied by the development of clamp-connexions at the cross-walls, and these outward and visible signs of diploidy have already made their appearance at *ddd* in the radial hyphae *cc*. Soon all the main radial hyphae will come to resemble *cc*, and then the whole mycelium will have changed from the haploid to the diploid state. An older mycelium which, as indicated by the development of clamp-connexions at all the cross-walls, is completely diploid is shown in Fig. 88.

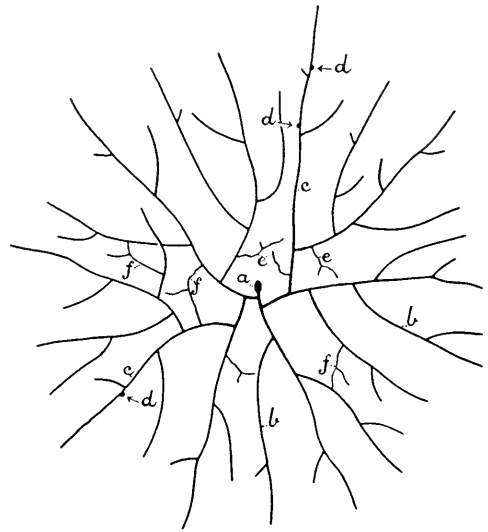


FIG. 87.—*Coprinus sterquilinus*, a homothallic Hymenomycete. Diagram of a young mycelium, to show the beginning of the production of clamp-connexions and of hyphal anastomoses: *a*, a basidiospore which has germinated; *b b*, leading hyphae growing radially away from the centre of the mycelium. On some of the leading hyphae *c c* clamp-connexions *d d* have now appeared, thus indicating that the mycelium is passing from the haploid to the diploid phase. Lateral hyphae *e e*, growing irregularly and more or less tangentially, are beginning to establish anastomoses in the older part of the mycelium, as at *f f*. Highly magnified.

Let us again turn to the mycelium illustrated in Fig. 87. At first all its hyphae had free ends; but already, as may be seen at *fff*, the end of a lateral hypha has grown toward and has fused with a radial hypha, so that the mycelium as a whole is becoming netted.

As such a young mycelium grows older, as shown in Fig. 88, the hyphal fusions become more and more numerous until at length the whole mycelium is converted into a closely-woven three-

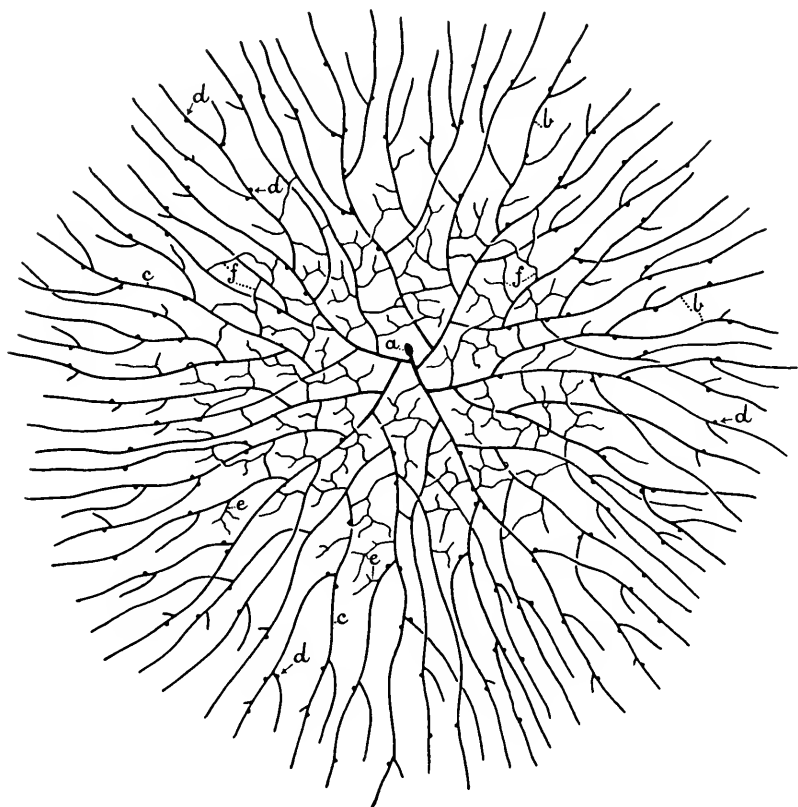


FIG. 88.—*Coprinus sterquilinus*, a homothallic Hymenomycete. Diagram of an older mycelium in the diploid phase: *a*, a basidiospore which has germinated; *b b* and *c c*, leading radial hyphae, now all bearing clamp-connexions *d d* at regular intervals; *e e*, lateral hyphae in the older part of the mycelium growing irregularly, many of them have established anastomoses as at *f f*, so that the mycelium is becoming converted into a closely meshed three-dimensional network. Highly magnified.

dimensional network. It may therefore be safely inferred that, when a mycelium has penetrated throughout a horse-dung ball, its hyphal fusions must number tens of thousands.

An older part of an actual mycelium of *Coprinus sterquilinus*, 0.15 square mm. in area, is shown in Fig. 89. Its hyphal fusions,

eleven in number, are all marked by arrows and, owing to the existence of these fusions, the various hyphae are connected together. At the rate of eleven hyphal fusions to each 0.15 square mm., a layer of mycelium like that shown in Fig. 89, but discoid and 6 cm. in diameter, would contain approximately 200,000 hyphal fusions. Clearly, therefore, if one wishes to understand the morphology and physiology of a mature mycelium of *C. sterquilinus*, it is necessary to grasp the fundamental fact that the mycelium is a fine-meshed network of hyphae extending in three directions of space.

The hyphal fusions which have just been described have nothing to do with sex; for (1) in homothallic Hymenomycetes, such as *C. sterquilinus*, the change from the haploid to the diploid condition is not necessarily preceded by any hyphal fusion whatever, and (2) hyphal fusions continuously increase in numbers long after the whole mycelium has become diploid, *i.e.* when the nuclei are already in pairs and are dividing conjugately.

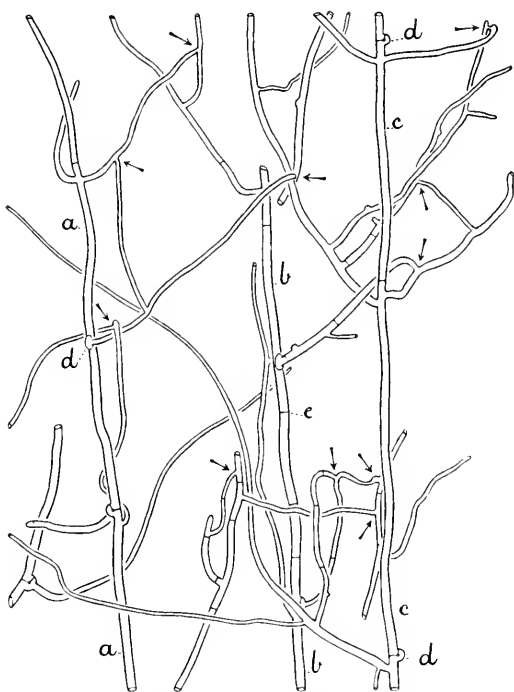


FIG. 89.—*Coprinus sterquilinus*, a homothallic Hymenomycete. Camera-lucida drawing of an older part of a mycelium in the diploid phase, growing in cleared dung-agar in a Petri dish. Mycelium seen in optical section. To show that the mycelium is a three-dimensional network: *a a*, *b b*, and *c c*, three hyphae which grew radially outwards from the centre of the mycelium. These hyphae, which bear clamp-connexions, as at *d d*, and also contain some plain septa, as at *e e*, are connected together by means of anastomoses effected by branch hyphae. There are eleven fusions to be seen in the drawing, and the position of each of them is indicated by an arrow. Area of mycelium, 0.15 square mm. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 187.

Let us now consider what happens when, in a single horse-dung ball, (1) only one spore germinates and (2) one hundred spores germinate.

(1) In Fig. 90 at A, as shown diagrammatically, there is a single spore in the middle of a small horse-dung ball. At B the spore has germinated and has formed a young mycelium which is growing outwards radially in all directions. At C the mycelium is older and now resembles the mycelium in Fig. 88: it has become diploid and hyphal fusions are taking place. At D the mycelium has penetrated to the exterior of the dung-ball and is now a highly complex diploid three-dimensional network. Moreover, it has formed a single fruit-body which as yet is only partially developed. Only one maturing fruit-body is shown at D because, as we have seen, a fruit-body of *C. sterquilinus* is so large that it often drains and completely exhausts all the mycelium in a single horse-dung ball.¹

What is happening to the fungus shown in Fig. 90, D? The fruit-body is growing at the expense of the cell-contents of all the hyphae of the mycelium. In other words, the contents of the mycelium are flowing toward the fruit-body from all parts of the horse-dung ball. Obviously, this flow is aided by the hyphal bridges which everywhere connect adjacent radial hyphae. Without the aid of these bridges the conduction to the fruit-body of the materials which are required for its growth would be a very slow and round-about process. Taking this for granted, it is easy to perceive that the significance of the hyphal fusions lies in part in the production of bridges which enable short cuts to be taken by materials flowing from the mycelium to the developing fruit-body. It has already been pointed out that the hyphal fusions in *Coprinus sterquilinus* have nothing to do with sex. We now see that they have a beautiful significance in connexion with the transfer of food materials from one place to another. It matters not at what particular spot on the exterior of the dung-ball the fruit-body

¹ The mycelium in any dung-ball like that represented in Fig. 90, D, always produces many minute fruit-body rudiments at the surface of the dung; but, as a rule, only one of these develops into a mature fruit-body, while the others all suffer abortion. For the sake of simplicity, aborted rudimentary fruit-bodies are not shown in Fig. 90, D.

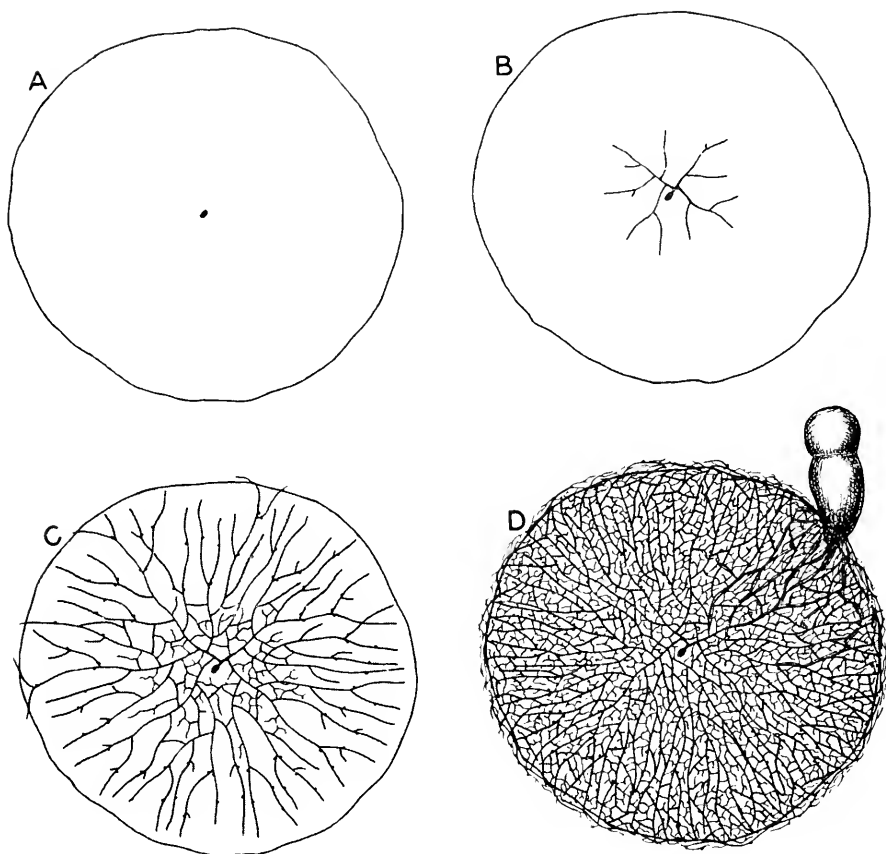


FIG. 90.—*Coprinus sterquilinus*, a homothallic Hymenomycete. Diagrams of a vertical section through a horse-dung ball containing a *single* spore which germinates and produces a mycelium that gives rise to a fruit-body. A, the spore in the middle of the dung-ball. B, the spore has germinated; the young mycelium is at present in the haploid state, for as yet its nuclei (as shown by special investigations) are isolated and non-conjugate, and it has not yet begun to produce clamp-connexions; also its branches have not yet begun to anastomose with one another. C, the mycelium, now larger and older, has passed from the haploid to the diploid phase, for its nuclei are now arranged in conjugate pairs as indicated by the fact that it bears clamp-connexions on all its leading radial hyphae; in the older part of the mycelium, lateral irregularly growing hyphae are anastomosing with one another and with the radial hyphae, so that the whole mycelium is becoming converted into a three-dimensional network. D, the mycelium, now about a month old, has everywhere become converted into a three-dimensional net-work and is exhausting itself by giving up its contents to a single large fruit-body. The fruit-body is developing at the expense of a single monosporous mycelium. The numerous hyphal fusions, which here can have no sexual or social significance, make possible the conveyance of food-materials to the fruit-body through numerous channels.

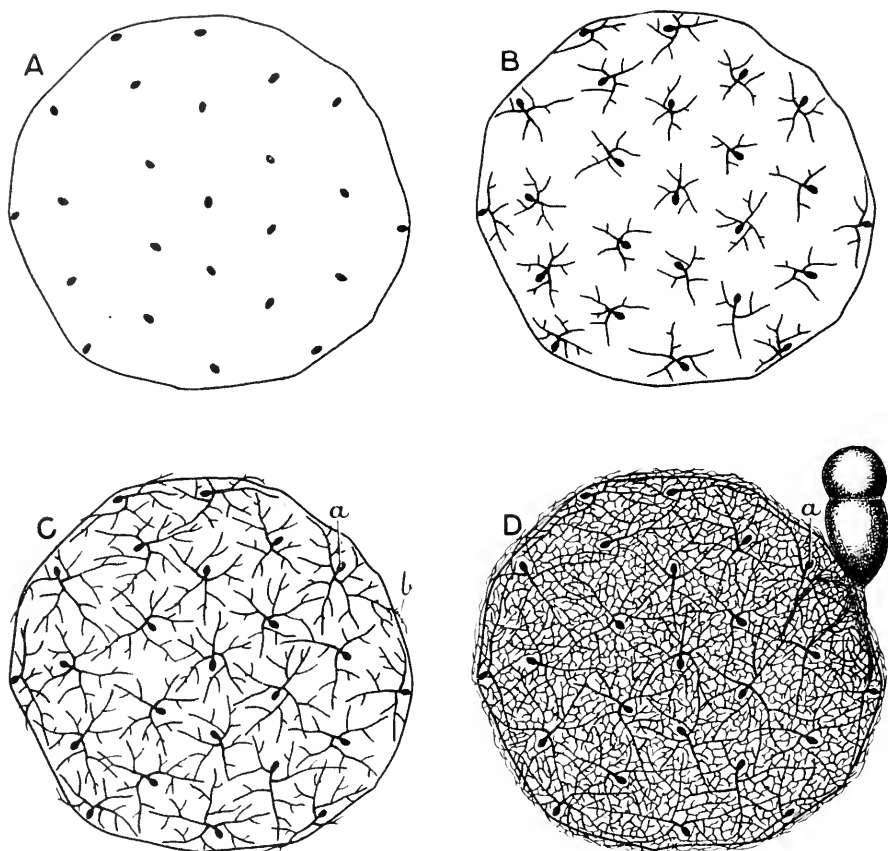


FIG. 91.—Social organisation of *Coprinus sterquilinus*, a homothallic Hymenomycete. Diagrams of a vertical section through a horse-dung ball containing numerous spores (24 shown) which germinate and produce mycelia which unite with one another and collectively exhaust themselves in promoting the development of a single fruit-body which has originated on one of the mycelia. A, the dung-ball, just dropped in a pasture : 24 spores are seen scattered within it at fairly uniform intervals. B, about 24 hours later ; all the spores have germinated and the young mycelia are rapidly invading the adjacent substratum. C, a few days later ; the mycelia are coming into contact with one another and are passing from the haploid to the diploid phase ; clamp-connexions are beginning to appear here and there, *e.g.* on the mycelium *a* ; the mycelia are about to unite with one another to form a compound three-dimensional network ; the hypha *b* of the mycelium *a* is destined to produce a fruit-body rudiment which will grow into a perfect fruit-body. D, about a month after the dung-ball was deposited ; the twenty-four monosporous mycelia in view, now in the diploid phase, have united to form a single, compound, closely-meshed, three-dimensional network. The fruit-body, which originated solely from the hypha *b* of the mycelium *a* and therefore contains nuclei derived from the mycelium *a* only, is developing at the expense not only of the mycelium *a* but also of all the other mycelia (23 others shown) which are yielding up their contents to it and are exhausting themselves in so doing. Not one of the simple monosporous mycelia by itself was large enough to produce a fruit-body ;

arises ; for, owing to the presence of the numerous hyphal bridges, there will always be plenty of channels leading to it.

The fruit-body shown in Fig. 90, D; finally elongates its stipe, expands its pileus, and discharges its spores. Then the whole of the mycelium is exhausted and no more fruit-bodies can be produced.

(2) Now let us suppose that, in a dung-ball of the same size as before, not one spore but one hundred spores are present. Let the spores be scattered uniformly in the dung-ball, as shown in Fig. 91, A. At B the spores have germinated and each spore has produced a young mycelium. At this stage of development the mycelia are competing with one another, for each mycelium is using up the food materials in its vicinity and, by radial growth, is invading fresh territory. Soon, however, as shown at C, the mycelia come near to one another peripherally. What happens next? After the individual mycelia have become diploid, or possibly before, hyphal fusions take place in each mycelium and between adjacent mycelia, so that the one hundred mycelia soon become converted into one single diploid closely-woven three-dimensional network. This *compound mycelium* in proceeding to reproduction, as shown at D, *behaves just like the single monosporous mycelium* already considered (cf. Fig. 91, D and Fig. 90, D) : it gives rise to a single large fruit-body. Again the hyphal bridges come into play, and the liquid contents of the mycelial hyphae flow from all directions toward the fruit-body, thus enabling it to complete its development ; and soon the fruit-body lengthens its stipe, expands its pileus, and liberates its spores.

The production of a single fruit-body of *Coprinus sterquilinus* from (1) a mycelium of *monosporous* origin and from (2) a mycelium of *polyporous* origin in two actual cultures, which were started on the same day, is shown in the photograph reproduced in Fig. 92.

FIG. 91—*cont.*

but, owing to the fact that all the mycelia have united and have acted together, it has been possible for a fruit-body to be produced on one of the mycelia, so that complete sterility of the whole group of mycelia has been avoided, with consequent benefit to the species as a whole. The numerous hyphal fusions, which here can have no possible sexual significance, are important in that they permit the various monosporous mycelia to act together socially in the production of a fruit-body and also in that they make possible the conveyance of food-materials to the fruit-body through numerous channels.

We know from the work of Brefeld¹ that, in the Coprini, every fruit-body comes into existence as a rudiment on a single

hypha (Figs. 93, 94, and 95). Now the hypha which gave rise to the rudiment which developed into the fruit-body shown in Fig. 91, D, could belong to only one of the one hundred mycelia. Therefore, what has happened in our compound mycelium is this: of the

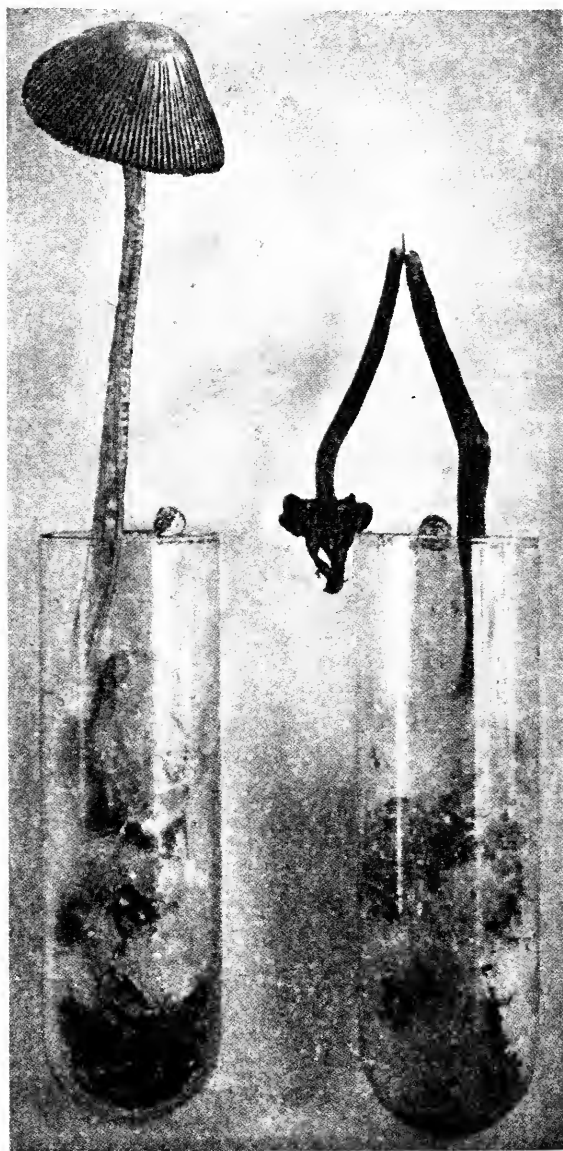


FIG. 92.—*Coprinus sterquilinus*, a homothallic Hymenomycete. On the left, a fruit-body developed from a simple mycelium derived from a single spore; on the right, a fruit-body developed from a compound mycelium derived from many spores. The cultures were started on the same day, about a month before the photograph was taken. The fruit-body on the right matured one day earlier than that on the left, and has now collapsed. Culture medium, sterilised horse dung. The cotton-wool plugs were removed from the tubes 48 hours ago to allow the fruit-bodies to elongate without being injured. Reduced to seven-tenths the natural size.

¹ O. Brefeld, *Untersuchungen über Pilze*, Leipzig, Heft III, 1877, pp. 33-35, Taf. I, Figs. 5 and 6.

one hundred united simple monosporous mycelia ninety-nine have given up the whole of their contents to one mycelium, thus enabling it to produce a fruit-body which may develop and liberate spores.

At first, perhaps, one might be disposed to consider that the one mycelium of the one hundred united ones which has produced a fruit-body in our hypothetical culture simply acts as a parasite upon the other ninety-nine. In a sense this parasitism must be admitted; for the favoured mycelium absolutely exhausts its fellows and carries out the business of developing a fruit-body which produces and liberates spores at their expense, but with its own cells and nuclei. But another explanation—that of social organisation in the interests of the species—seems preferable to the parasitic one.

The social explanation of the facts illustrated in Fig. 91 may be stated as follows. For the production of so large a structure as a fruit-body of *Coprinus sterquilinus* a very considerable amount of mycelial contents is required. To obtain this amount of mycelial contents a single simple monosporous mycelium must become master of a certain minimum mass of the nutrient substratum—a small dung-ball or a mass of dung equivalent thereto. But for a monosporous mycelium to obtain mastery of so much of the substratum under natural conditions must often be difficult or impossible to accomplish owing to competition from other species of fungi; and perhaps, when there is only a single spore of *C. sterquilinus* in a dung-ball, the mycelium which proceeds from it but rarely succeeds in becoming massive enough to form a fruit-body. However, in a single dung-ball, at the moment of its deposition, there must often be a large number of spores of *C. sterquilinus* embedded.¹ Let us suppose, as already done in (2), that in one particular dung-ball one hundred spores of *C. sterquilinus* are so embedded that they are equally distributed throughout the ball and that all of them germinate and give

¹ A large fruit-body of *Coprinus sterquilinus* may produce as many as 100,000,000 spores (Vol. III, p. 223). In a pasture many fruit-bodies may come up on horse-dung plats. The spores are carried by the wind on to the herbage, to the leaves and stems of which they cling with great tenacity (Vol. III, p. 229). A horse when grazing must often swallow thousands or even tens of thousands of them. They pass down the alimentary canal unharmed, so that all of them are extruded in the faeces.

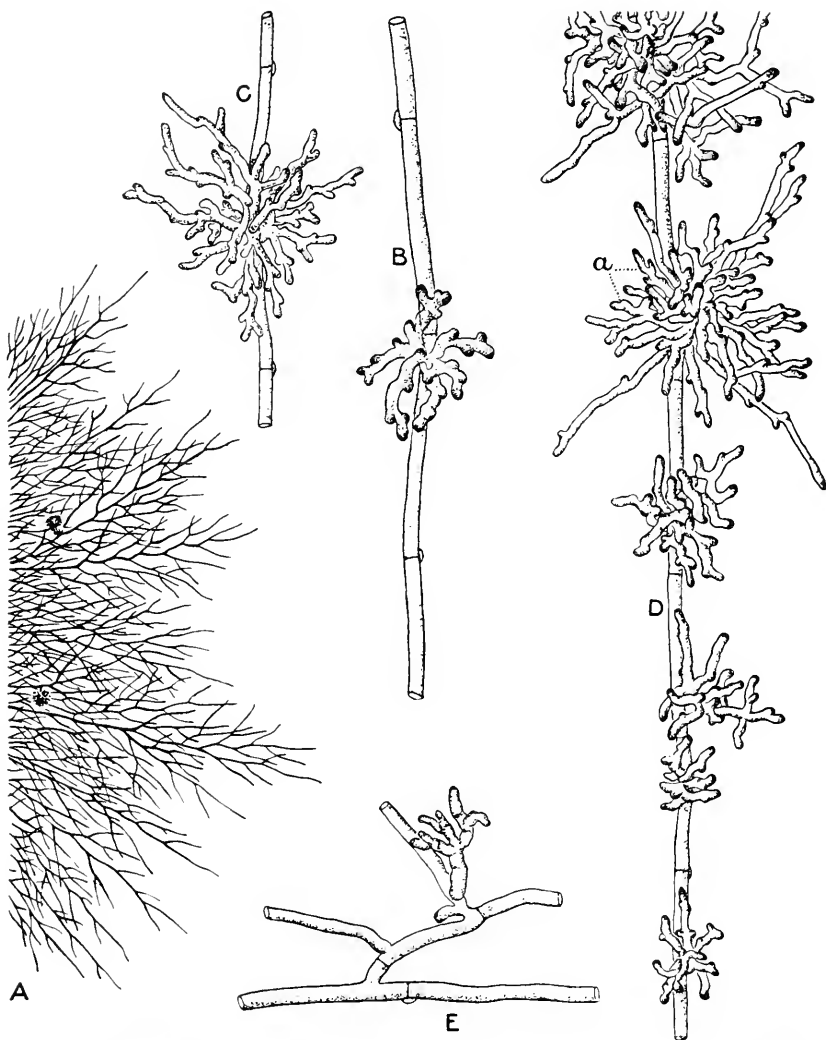


FIG. 93.—*Coprinus stercorearius*. To show the origin of a fruit-body from a single cell of the mycelium. The species is homothallic, so that each young monosporous mycelium soon passes, without union with any other mycelium, from the haploid to the diploid phase. A : part of a diploid mycelium derived from a single spore, growing in a dung decoction, now giving rise to fruit-bodies ; two of these fruit-bodies which are very young but already have the pileus well differentiated are represented ; each of the fruit-bodies has arisen from a single diploid cell. B : part of a leading radial hypha of a mycelium like that shown at A ; it is divided into cells by three septa, each of which is provided with a clamp-connexion (the clamp-connexion of the middle septum presumably is hidden) ; one of the two entire cells represented, near one of its ends, has given rise to a fruit-body rudiment which consists of a cluster of several irregular hyphae. C : the same as B, but less highly magnified and with the fruit-body rudiment in a

rise to mycelia (*cf.* Fig. 91, A and B). If now these one hundred mycelia were all to develop equally well and independently of one another, no single one of them would be large enough to give rise to a fruit-body of even the minimum size. *All the mycelia would remain sterile.* But what actually happens where one hundred mycelia begin their development simultaneously in the same dung-ball is that, as already explained, the mycelia become united by hyphal fusions into a three-dimensional network of very fine mesh. *Competition thus becomes converted into co-operation*, with the result that general sterility is replaced by successful reproduction: a single large fruit-body is produced at the expense of the whole compound mycelium which is one hundred times more extensive than any of its components.

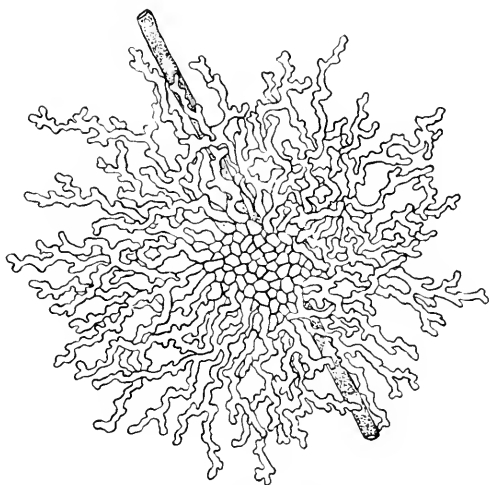


FIG. 94.—*Coprinus stercorarius*. To show the origin of a fruit-body from a single cell of the mycelium. A mycelial hypha (shaded) lies in the background; one of its cells has given rise to a fruit-body rudiment which is more advanced in development than those shown in Fig. 93. The rudiment has been flattened out in the culture medium by pressure on the cover-glass so as to disclose its structure. Its inner more compact part will develop into the stipe and pileus, and its outer more irregular hyphae into the volva. Magnification, 338. The drawing copied by Dr. Nellie Carter from Brefeld's *Taf. II* in *Heft III* of his *Untersuchungen über Pilze*, and reproduced on a slightly larger scale.

The reproductively unsuccessful mycelia of the compound mycelial network of *Coprinus sterquilinus* illustrated in Fig. 91, D, are analogous to the neuters in a bee-hive that toil with all their

FIG. 93—*cont.*

more advanced stage of development. D: another leading radial hypha of a mycelium like that shown at A, consisting of a series of cells each of which has given rise to a fruit-body rudiment; only one of these rudiments, namely, that marked *a*, eventually developed into a perfect fruit-body, while all the others became aborted. E: a fruit-body rudiment which has arisen on a secondary branch of a mycelium and not on a leading radial hypha. Magnification: A, 11·25; B and E, 395; C and D, 225. The drawings copied by Dr. Nellie Carter from Brefeld's *Taf. I* in *Heft III* of his *Untersuchungen über Pilze*, and reproduced on a slightly larger scale.

life's energy to collect the food that is destined to contribute to the development, not of their own descendants, but of those of the queen bee and of one of the drones. The neuter bees die of exhaustion brought on by their foraging expeditions; but their sacrifice is not in vain, for it is obviously indispensable for the welfare of the hive as a whole and a prime factor in securing the persistence of the hive-bee species, *Apis mellifica*, from generation

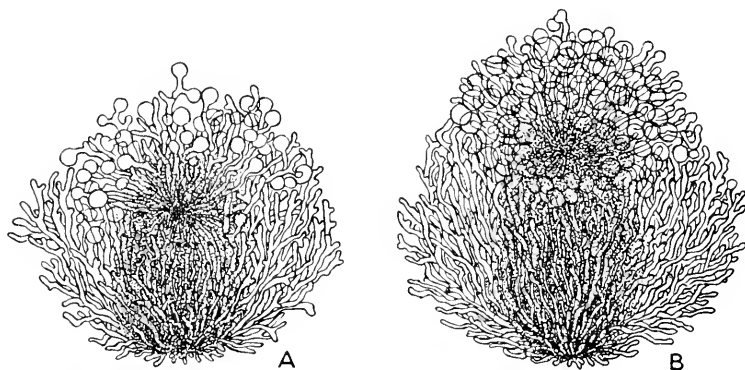


FIG. 95.—*Coprinus stercorearius*. Lateral view of two young fruit-bodies each of which developed from a single cell of a mycelial hypha. The hypha at their base has not been represented (cf. Fig. 94). B is slightly more advanced in development than A. In both may be distinguished the following parts: the core of the stipe (shaded); the core of the pileus (more deeply shaded); the volva around the stipe (free hyphae directed upwards); and the volva around the pileus (hyphae breaking up into rounded cells which eventually will form a mealy covering to the pileus when the fruit-body is mature). Magnification, 135. The drawings copied by Dr. Nellie Carter from Brefeld's Taf. II in Heft III of his *Untersuchungen über Pilze*, and reproduced on a slightly larger scale.

to generation. Only one of the one hundred united mycelia of *C. sterquilinus* in a single dung-ball produces a fruit-body, and the other ninety and nine remain sterile and completely exhaust themselves in supplying the more fortunate mycelium with the necessary food-stuffs; but, here again, the sacrifice is not in vain, for it is of the greatest advantage to the species: it enables reproduction to take place where otherwise there would be nothing but sterility, and it thus contributes to the persistence of the species in successive generations.

The social organisation of *Coprinus sterquilinus* has been compared with that of the hive-bee in that certain individuals do not

exercise any reproductive function, but merely assist those which do. However, the neuters of *Apis mellifica* and the non-reproducing mycelia in a compound mycelium of *Coprinus sterquilinus* exhibit marked differences. A neuter bee has a structure strikingly different from that of a queen bee or a drone, which prevents the neuter bee from reproducing itself but admirably fits it for the work that it has to do; whereas, in the compound mycelium of the *Coprinus*, the individual mycelia are morphologically indistinguishable. The mycelium which produces a fruit-body does so by virtue of its more favourable position in the substratum and not by virtue of superior reproductive power. The factors, such as light, space, etc., which decide where on the exterior of a dung-ball a fruit-body rudiment may develop into a perfect fruit-body have already been treated of in Part I, Chapter V.

Social Organisation in Other Hymenomycetes.—Whenever the mycelium of one of the Hymenomycetes is studied in detail in artificial cultures, the hyphal fusions which are formed between adjacent hyphae of the same monosporous mycelium, or between hyphae of different monosporous mycelia, are almost sure to attract attention. Brefeld¹ records great numbers of hyphal fusions as occurring in monosporous mycelia of *Coprinus stercorarius* and points out that, in this species, even very young mycelia, when scarcely more than germ-tubes, often become united into a single network. Richard and Olga Falck,² in the account of their study of the germination of the spores of *Psalliota campestris*, show hyphal fusions in a monosporous mycelium and also several mycelia uniting together to form a single compound network. I, myself, have observed hyphal fusions similar to those just described not only in *Coprinus sterquilinus* but in many other Coprini, particularly in *Coprinus lagopus*, and also in *Panus stypticus*. For *Coprinus lagopus*, a heterothallic species of which fruit-bodies are shown in Fig. 48 (p. 76) and in Figs. 107, 108, and 109 (*vide infra*), the union

¹ O. Brefeld, *Untersuchungen über Pilze*, Leipzig, Heft III, 1877, pp. 16–17, Taf. I, Fig. 3. Brefeld also observed anastomoses in the mycelia of *Coprinus lagopus*, *C. ephemerus*, and *C. ephemeroides* (*ibid.*, pp. 103, 110, 118).

² Richard and Olga Falck, “Über die Sporenkeimung des Champignons,” in Falck’s *Mycologische Untersuchungen und Berichte*, Beiheft 1, 1924, Text-figs. 1 and 5.

of three haploid mycelia, each derived from a single basidiospore,

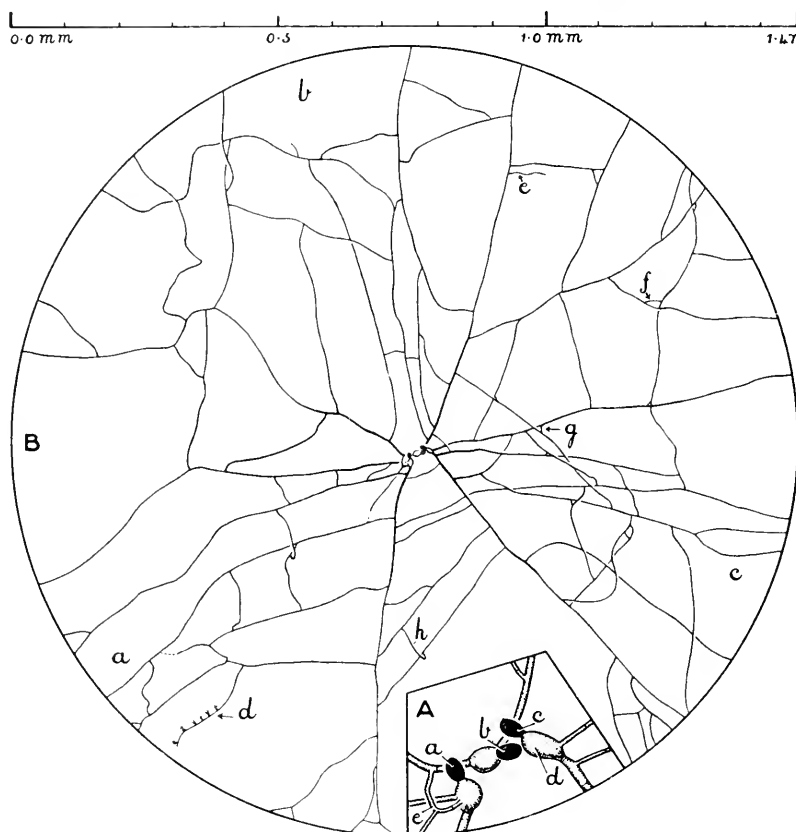


FIG. 96.—*Coprinus lagopus*. Union of three monosporous mycelia to form a compound mycelium. Drawing made nine days after sowing the spores in a shallow drop of horse-dung fluid. A, three basidiospores *a*, *b*, and *c* which have germinated and produced three mycelia; *d*, a germ-tube vesicle; *e*, a hypha uniting the mycelium produced by the spore *a* with that produced by the spore *b*. B, the three spores of A and their mycelia shown on a smaller scale: the spores *a*, *b*, and *c* have given rise to three mycelia *a*, *b* and *c* respectively; *d*, a hypha bearing oidiophores and oidia; the three monosporous mycelia have become united to form a single, finely netted, compound, haploid mycelium; most of the lateral hyphae have grown toward, and have met and fused with, another hypha, and freely-ending hyphae, as at *e*, are few in number; where two hyphae have crossed near to one another, as at *f* and *g*, a bridging hypha has been put out from one to the other; in some places, as at *h*, a hypha has crossed over another hypha and has fused with it after making a growth-curvature through 180°. Magnification: A, 293; B, 110.

into a single compound mycelium is shown in Fig. 96. This mycelium grew in a shallow drop of horse-dung water and the

limited food-supply may have been a factor tending to increase the number of hyphal fusions. When the drawing was made, very few freely-ending hyphae, such as the one shown at *e*, could be found. The union of a diploid mycelium with a haploid mycelium of *C. lagopus* is shown in the next chapter in Fig. 135. The union of two diploid mycelia of *Panus stypticus* is illustrated in Volume III, Fig. 176 (p. 415). Falck,¹ in his extended study of the destruction of wood by species of *Lenzites* observed that in *Lenzites* hyphal anastomoses are characteristic both of the primary mycelium—which he calls the *netzmycel*—and of the secondary mycelium; and, in an illustration of the mycelium of *Lenzites abietina* present in the tracheides and medullary-ray cells of *Abies alba*, he represents the hyphae as being joined into a network. Doubtless, under natural conditions, many spores of one and the same hymenomycetous species often settle close together on the same organic substratum, such as wood, dead leaves, etc., and germinate side by side. Wherever this happens, it is probable that the monosporous mycelia join together to form a single compound net-mycelium which acts as a unit in the formation of one or more fruit-bodies. There is therefore good reason to suppose that social organisation, like that described in *Coprinus sterquilinus*, is characteristic of the Hymenomycetes in general.

Recent studies on sex in the Hymenomycetes indicate that most Hymenomycetes are heterothallic, *i.e.* that two monosporous mycelia of opposite sex must unite and their nuclei become conjugate, before a normal diploid fruit-body can be developed. Among these heterothallic species may be mentioned: *Coprinus lagopus*,² *Panaeolus campanulatus*,³ *Collybia velutipes*,⁴ *Marasmius oreades*,⁵ *Schizophyllum commune*,⁶ and *Fomes pinicola*.⁷ In view of our knowledge of social organisation in the Hymenomycetes, we may be sure that, under natural conditions, single fruit-bodies of these and other heterothallic Hymenomycetes are not always the product merely of the two mycelia of opposite sex from which their nuclei

¹ R. Falck, "Die *Lenzites*-Fäule des Coniferenholzes," in Möller's *Hauschwammforschungen*, Jena, Heft III, 1909, pp. 102, 104, Taf. V, Fig. 4.

² Irene Mounce (1921).

³ R. Vandendries (1923).

⁴ F. Zattler (1924).

⁵ In my own laboratory, hitherto unpublished.

⁶ H. Kniep (1920).

⁷ Irene Mounce (1926).

are derived, but are often the product of a compound mycelium made up of many mycelia, the number doubtless varying from a few to many thousands.

Social Organisation in Other Fungi.—So far as social organisation is concerned, in all probability the Gastromycetes behave like the Hymenomycetes, a single fruit-body often being produced by the joint efforts of many united mycelia.

Among the Ascomycetes, hyphal fusions have been observed in many species of Pyrenomycetes and Discomycetes. De Bary,¹ in



FIG. 97.—Germinating conidia of *Nectria Solani* Reinke: *a*, developing by itself; the others have anastomosed so as to form several compound mycelia. Drawn by A. de Bary; reproduced from Fig. 1 of his *Vergleichende Morphologie und Physiologie der Pilze*, 1884. Magnification, 390.

his well-known text-book, stated that the coalescence of two hyphae may take place even when they belong to mycelia which have sprung from different spores, and he illustrated his remark with a drawing showing eight conidia of *Nectria (Spicaria) Solani* connected together by their germ-tubes (Fig. 97). The brothers Tulasne observed and illustrated similar unions in *Cryptospora aucta*,² *Pleospora herbarum*,³ *Hypocrea rufa*,⁴ *Hypomyces rosellus*,⁵ and *Nectria Stilbosporae*.⁶ Woronin⁷ has provided us with a drawing showing

anastomoses between several young mycelia derived from the ascospores of *Sordaria fimiseda*. The same author⁸ observed the fusion of one hypha with another and the formation of bridges between parallel hyphae in the mycelium of *Ascobolus pulcherrimus*, and Brefeld⁹ observed numerous hyphal fusions and

¹ A. de Bary, *Vergleichende Morphologie und Biologie der Pilze*, Leipzig, 1884, p. 2, Fig. 1.

² L.-R. and C. Tulasne, *Selecta Fungorum Carpologia*, Parisiis, Tomus II, 1863, Tab. XVII, Figs. 3 and 4.

³ *Ibid.*, Tab. XXXII, Fig. 4.

⁴ *Ibid.*, Tomus III, Tab. III, Fig. 8.

⁵ *Ibid.*, Tab. V, Fig. 3.

⁶ *Ibid.*, Tab. XI, Fig. 16.

⁷ M. Woronin, in de Bary and Woronin's *Beiträge zur Morphologie und Physiologie der Pilze*, Frankfurt a. M., Dritte Reihe, 1870, Tab. IV, Fig. 1.

⁸ *Ibid.*, Zweite Reihe, 1866, Tab. II, Figs. 9 and 10.

⁹ O. Brefeld, *Untersuchungen über Pilze*, Leipzig, Heft IV, 1881, Taf. IX, Fig. 14.

the formation of a closely-meshed network in the mycelium of *Peziza Sclerotiorum*. In all these Ascomycetes, and in many others, the union of a number of relatively weak and otherwise competing mycelia, with the formation of a single strong compound mycelium in which the units may co-operate in the production of one or more fruit-bodies, must often be of considerable advantage in the struggle for existence.

The occurrence of hyphal fusions and of social organisation in the Pyrenomycetes may be illustrated by some observations of my own on the conidial stage of *Hypocrea rufa*. This conidial stage, as was proved by the anatomical studies of Tulasne¹ and by the cultural experiments of Brefeld,² is nothing more or less than the well-known fungus *Trichoderma lignorum*, which for systematic purposes is usually placed in the Fungi Imperfecti. *Trichoderma lignorum* forms rounded, somewhat lens-shaped, green, conidial mats on dead bark, wood, leaves, and other wet and decaying plant remains in both Europe and North America, more especially in late autumn and winter.

Trichoderma lignorum invaded a large sterilised horse-dung culture, which had been prepared for the cultivation of *Coprinus sterquilinus*, and grew there luxuriantly so that, eventually, it spread over the entire surface of the dung. I sowed several hundreds of the green conidia in a hanging drop of horse-dung juice. The spores swelled up and germinated, and the germ-tubes or young monoconidial mycelia all became united into a single network which was

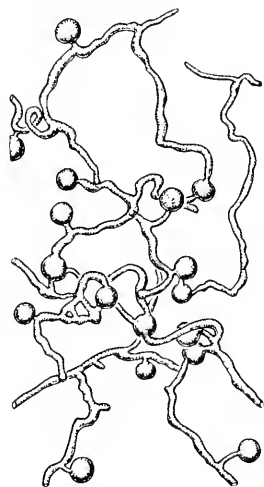


FIG. 98.—*Trichoderma lignorum*. Union of mycelia derived from different conidia. Conidia placed in a drop of dung-agar two days previously; the germ-tubes have fused with one another and with the conidia, so that a compound netted mycelium has been formed. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 467.

¹ L.-R. and C. Tulasne, *loc. cit.*, Tomus III, Tab. III, Fig. 9.

² O. Brefeld, *loc. cit.*, Heft X, 1891, p. 190, Taf. V, Figs. 56 and 57. Brefeld sowed the ascospore-halves of *Hypocrea rufa* and, after eight days, the mycelium so produced developed the typical, branched, more or less pyramidal conidiophores and the green conidia of *Trichoderma lignorum*.

immersed in the liquid (*cf.* Fig. 98). Under these aquatic conditions no spores were formed. However, a hypha of one of the united monoconidial mycelia, which happened to be at the edge of the drop, managed to push its way out of the liquid and to grow into the air. The aerial hypha grew in length and branched and rebranched so as to form a large conidiophore, and soon at the end of each branch it produced a little ball of green conidia. With the microscope I was able to observe that, during the formation of the conidiophore and its spores, the compound mycelium gradually became exhausted of its contents, so that at last the hyphae seemed to be empty. There can be no doubt that the compound mycelium, in supplying food materials to the conidiophore, acted as a unit, and that every one of the monoconidial mycelia of which it was made up contributed something to the formation of the spores. Of the hundreds of mycelia which were formed by the germination of the conidia soon after the culture was started, only one succeeded in carrying out the process of reproduction, and it developed its conidia to some extent by drawing upon its own contents, but chiefly at the expense of the contents of all the other mycelia which remained sterile. The co-operation of the individual mycelia with one another led to the production of far more spores than could have been produced by competition. The reproductively successful mycelium owed its success to its position in the medium: it happened to be near the edge of the drop and therefore had the good fortune to push one of its hyphae beyond the edge of the drop into the air, whereas all the other mycelia happened to be farther within the drop and to remain totally immersed in the watery fluid where, owing to the inhibitory action of the water, the production of conidia was impossible. The parallel between the social organisation which has just been described for *Trichoderma lignorum* and that previously described for *Coprinus sterquilinus* is very striking. In both the Mould and the Hymenomycete most of the co-operating individual mycelia remain sterile, while only one or a very few of them succeed in producing spores, but the species benefits in that the spores are produced with greater certainty and in greater numbers.

Further observations on social organisation in *Trichoderma*

lignorum, confirming those just described, were made by the author with the assistance of Miss Ruth Macrae in the autumn of 1927. Some conidia obtained from a green conidial mat found on rotting wood at Winnipeg were sown in a hanging drop of malt-agar. They germinated readily and each one gave rise to a mycelium

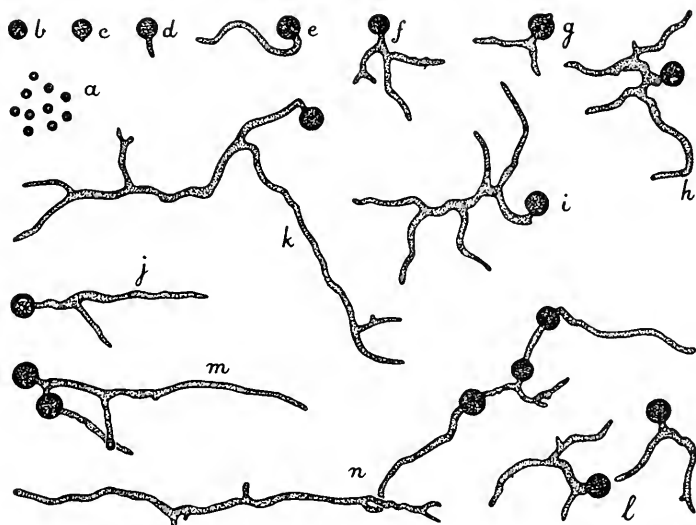


FIG. 99.—*Trichoderma lignorum*. Germination of spores (conidia) on malt-agar and the non-sexual fusion of monosporous mycelia: *a*, spores just placed in the medium; *b-l*, drawn 16-17 hours later; *m* and *n* drawn 24 hours later; *b*, a swollen spore; *c*, *d*, *e*, spores developing germ-tubes; *f-k*, young monosporous mycelia; *l*, two monosporous mycelia, the one on the right by means of a hypha is about to touch and to unite with the swollen spore of the other; *m* and *n*, two compound mycelia, formed by the fusion of two and of three simple monosporous mycelia respectively. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 466.

filled with fine protoplasm (Fig. 99, *a-k*). Where two or more mycelia were formed close together in the medium, they soon fused (Fig. 99, *l-n*). In one instance (Fig. 100) seven spores produced as many simple monosporous mycelia which fused together to form one large compound mycelium. This compound mycelium was entirely submerged in the agar medium. Ultimately, one of the monosporous mycelia (Fig. 100, *a*), which happened to be near the surface of the agar, gave rise to a conidiophore (*b*) which pushed its way into the air and produced balls of green conidia (*c*). The

conidia of each ball were held together by a drop of liquid. At the stage shown in Fig. 100, the peripheral parts of the compound



FIG. 100—Social organisation in *Trichoderma lignorum* made possible by non-sexual hyphal fusions between monosporous mycelia. Seven spores (conidia), *s s*, sown in a hanging drop of malt-agar, have produced seven monosporous mycelia which have fused together to form one compound mycelium. Only one of the monosporous mycelia, *a*, has produced a conidiophore, *b*, and balls of conidia, *c*. The other six mycelia are remaining sterile and are giving up their contents to *a*. The peripheral parts of the compound mycelium, such as *d*, *e*, *f*, and *g*, have already lost most of their protoplasmic contents (for full mycelia cf. Fig. 99). The compound mycelium is entirely submerged in the malt-agar, the conidiophores and conidia are sub-aerial. The conidia in each ball are held together by a drop of liquid. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 466.

mycelium, (*d*, *e*, *f*, *g*), have already become emptied of most of their protoplasmic contents. From an inspection of Fig. 100 it is obvious

that of the seven monosporous mycelia which have fused together, one only is carrying on the work of reproduction at the expense of the protoplasm of all the seven mycelia. Thus, six of the monosporous mycelia, while themselves sterile, are assisting the seventh in carrying out its reproductive function.

Another Pyrenomycete in which hyphal fusions may be readily observed is *Pleurance anserina* (Fig. 101) which often comes up spontaneously and fruits very freely upon unsterilised horse-dung balls in laboratory cultures at Winnipeg. Miss Dowding,¹ working under my direction, found that normally there are four binucleate bisexual spores in each ascus but that, occasionally, a single normal spore is replaced by two uninucleate unisexual dwarf spores. When a number of normal spores or two dwarf spores are sown together in a drop of the culture medium, the mycelia which they produce soon become united together in



FIG. 101.—*Pleurance anserina*. On the sterilised horse dung in the glass dish is a mycelium which originated from a single normal ascospore. The mycelium is about two weeks old and has fruited abundantly. The perithecia are scattered over the surface of the culture medium and their black necks can be seen protruding above the white mycelium. The asci usually contain four binucleate bisexual spores but, occasionally, one of these normal spores is replaced by two dwarf spores each of which is uninucleate and unisexual. Photographed by A. H. R. Buller and E. Silver Dowding. Natural size.

many places, so as to form a single network. The union of two mycelia derived from two dwarf spores is illustrated in Fig. 102.

To those Discomycetes in which monosporous mycelia unite to form a single compound mycelium may be added *Ascobolus magnificus*, the very large fruit-bodies of which are illustrated in Fig. 103. A number of ascospores of a fruit-body of this species were shot

¹ E. Silver Dowding, "The Sexuality of the Normal, Giant, and Dwarf Spores of *Pleurance anserina* (Ces.) Kuntze," *Annals of Botany*, vol. xlv, January, 1931.

on to cleared dung-agar in a Petri dish. Here many of them germinated within twenty-four hours. The monosporous mycelia

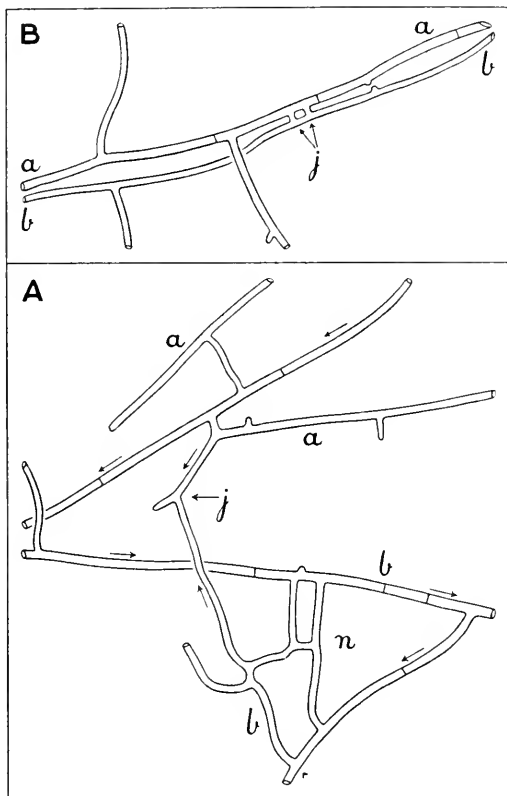


FIG. 102.—*Pleurage anserina*, one of the Pyrenomyces. To show hyphal anastomoses between mycelia derived from different dwarf spores. A, the mycelium *a a* was derived from one dwarf spore and the mycelium *b b* from a dwarf spore of opposite sex. The mycelium *b b* in the region *n* has become netted, and one of its hyphae has fused with a hypha of the mycelium *a a* at *j*. The arrows along the hyphae indicate the direction of growth. B, similar to A: a hypha of the mycelium *a a* has become united to a hypha of the mycelium *b b*; the junction has been effected at *j* by means of short bridging hyphae. Drawn by A. H. R. Buller and E. Silver Dowing. Magnification, 400.

soon came into contact and fused with one another, so that a fine-meshed compound mycelium was eventually formed. Part of this mycelium is shown in Fig. 104. In the formation of a large fruit-body on horse dung in the field under natural conditions it is probable that the mycelium is often, or even usually, compound. Doubtless in such a compound mycelium, while two monosporous mycelia only are concerned with the sexual phenomena leading to the production of the fruit-body (the species is heterothallic), the other monosporous mycelia forming part of the compound mycelium contribute socially to the development of the fruit-body and to the production of its spores by yielding up to the fruit-body their nutritive contents.

As an example of the existence of hyphal fusions in one of the Fungi Imperfecti in which the perfect fruiting stage is unknown

may be mentioned *Colletotrichum trichellum* (Fr.) Duke (= *Vermicularia trichella* Fr.), a species studied by Miss Duke, to whom the author is indebted for Fig. 106. Miss Duke¹ sowed many spores of the *Colletotrichum* in a hanging drop of water and observed that



FIG. 103.—*Ascobolus magnificus*. Surface of a sterilised horse-dung culture, showing a number of large fully-expanded fruit-bodies in which spore-discharge was in progress. Photographed by B. O. Dodge. Natural size.

the young mycelia freely joined with one another so as to form a mycelial network (Fig. 106, C, D, and E). It seems likely that, under natural conditions, the acervuli (Fig. 105), which develop on the leaves of the Ivy (*Hedera Helix*), often originate from a

¹ The hyphal fusions here under consideration were observed in the course of a systematic study of the old genus *Vermicularia*. Cf. Maud M. Duke, "The Genera *Vermicularia* Fr. and *Colletotrichum* Cda," *Trans. Brit. Myc. Soc.*, vol. xiii, 1928, pp. 156-184.

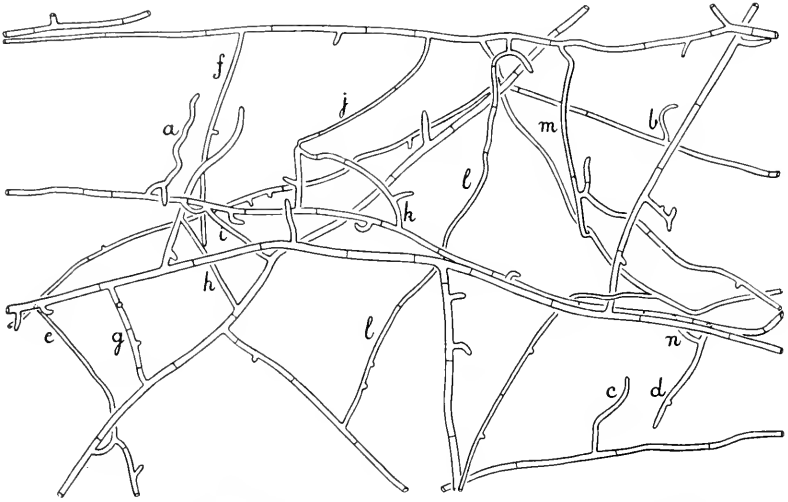


FIG. 104.—*Ascobolus magnificus*, one of the Discomycetes. Camera-lucida drawing, to show hyphal anastomoses. A number of ascospores were shot on to cleared dung-agar in a Petri dish where they germinated within 24 hours. The monosporous mycelia united to form a three-dimensional network, part of which is here shown. Drawing made eight days after sowing the spores: *a, b, c, and d*, hyphae which are elongating and which later would probably fuse with other hyphae; *e and f*, two hyphae each with its free end in contact with, and probably about to fuse with, another hypha; *g, h, i, j, k, l, m, and n*, eight hyphae which have anastomosed with other hyphae and therefore have contributed to the transformation of the mycelium into a three-dimensional network. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 120.

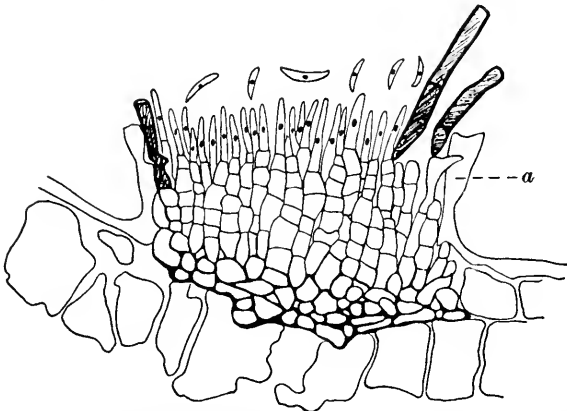


FIG. 105.—*Colletotrichum trichellum* (Fr.) Duke (= *Vermicularia trichella* Fr.). Transverse section of one of the acervuli in a leaf-spot of an Ivy leaf (*Hedera Helix*). The acervulus is mature. The cuticle of the leaf, *a*, has been ruptured by the setae, and conidia are being produced on the conidiophores. Where many conidia germinate on a leaf and infect it, the mycelia doubtless fuse together and co-operate in the formation of acervuli. Drawn by Maud M. Duke (*Trans. Brit. Myc. Soc.*, Vol. XIII, 1928, p. 162). Magnification, about 445.

compound mycelium and are therefore the product of a social organisation.

The Various Functions of Hyphal Fusions in the Hymenomycetes.—Hyphal fusions in the Hymenomycetes are of importance in the five following ways : (1) they convert every young mycelium

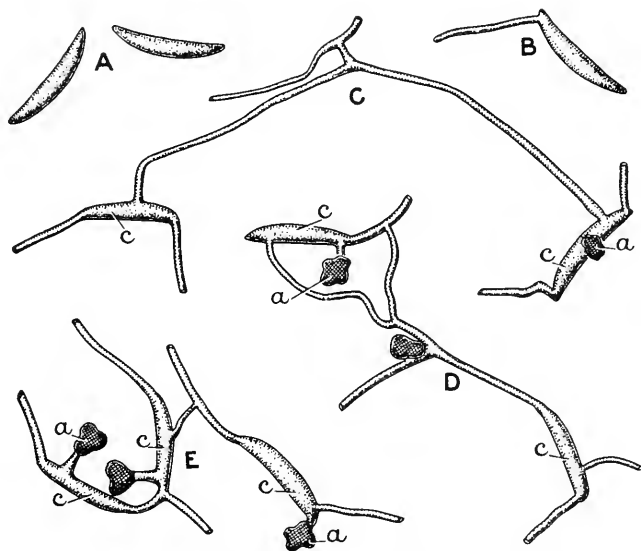


FIG. 106.—*Colletotrichum trichellum* (Fr.) Duke (= *Vermicularia trichella* Fr.). To show fusion of germ-tubes or young mycelia derived from two or more conidia. A, two conidia. B, a conidium germinating. C, two young mycelia, derived from two separate conidia *c c*, which have anastomosed to form a single compound mycelium ; *a*, an appressorium. D, similar to C, but there is a triangular mesh above and two appressoria *a* are present. E, three conidia *c c c* have been connected by hyphal fusions effected by their germ-tubes ; each conidium has sent out a short hypha, the end of which has formed an appressorium *a*. From original drawings made for the author by Maud M. Duke. Magnification, 608.

into a network of such a kind that food materials can be conducted through it to fruit-bodies, sclerotia, etc., in diverse directions ; (2) in heterothallic species, they place two mycelia of opposite sex in continuity, thus making possible the association of their nuclei in conjugate pairs ; (3) in heterothallic species, they convert the haploid mycelia into a network of such a kind that, when a haploid mycelium is being diploidised by nuclei derived from another haploid mycelium, the nuclei can travel through the mycelium

which is being diploidised by numerous and varied routes ; (4) they convert all mycelia, whether haploid or diploid, into a network of such a kind that a mycelium, when injured by the breaking of some of its hyphae, still remains a unit and can act as such in the production of a fruit-body ; and, finally, (5) in any single species, whether homothallic or heterothallic, they permit of any number of adjacent mycelia, whatever may be their sexual state, uniting to form a compound mycelium and thus acting as a social unit in the production of fruit-bodies and spores. A brief discussion of the five functional advantages accruing to hymenomycetous mycelia as a result of the formation of hyphal fusions will now be attempted.

(1) *Conduction of food materials.* A fruit-body of *Coprinus sterquilinus* or any other Hymenomycete, or a sclerotium of *Coprinus stercorarius*, etc., is produced entirely at the expense of the vegetative mycelium, and all the food materials required for its development pass along mycelial hyphae to it. The exact place on a mycelium where a fruit-body or a sclerotium is to develop to maturity is decided by external conditions. It is important, therefore, that, when a fruit-body or a sclerotium begins its development, wherever it may be situated it shall be able to receive the contents of the vegetative mycelium without difficulty. A large vegetative mycelium without any hyphal fusions and entirely un-netted would be about as unfitted for conducting food materials to a large fruit-body as a single un-netted railway system would be for carrying goods and passengers in a country such as England. A finely-netted mycelium provides many different channels through which food materials can be conducted to a developing fruit-body or sclerotium and therein lies a very important part of its physiological significance.

(2) *Mating of mycelia.* In a heterothallic species, e.g. *Coprinus lagopus*, it is obvious that, if two haploid mycelia are to interact sexually, somehow or other they must unite. The union is actually accomplished by one or more hyphal fusions. Theoretically, a single hyphal fusion between two haploid mycelia which are able to mate with each other might be sufficient to allow of an interchange of nuclei which would initiate the diploidisation process in the two mycelia ; but, actually, when two mycelia of opposite

sex mate with each other, as may be seen in plate cultures, they unite with each other at a number of points, so that doubtless the exchange of nuclei takes place across many bridges. The formation of numerous hyphal fusions between mating mycelia therefore assists the beginning of the sexual process.

(3) *Passage of nuclei through a haploid mycelium becoming diploidised.* As will be shown in the next chapter, in *Coprinus lagopus*, when a large haploid mycelium of one sex is being diploidised by nuclei derived from an inoculum consisting of a tiny haploid mycelium of opposite sex, the nuclei move through the large mycelium in all directions and the movement ends by the establishment of pairs of conjugate nuclei (indicated by clamp-connexions) in all the leading hyphae of the large haploid mycelium. The rapid diploidisation of the large haploid mycelium by means of nuclei derived from the tiny inoculum would be impossible were it not for the fact that the large haploid mycelium is a finely-meshed three-dimensional network of hyphae. The nuclei do not need to go to the centre of the mycelium and then radially outwards to all the thousands of branches; but, owing to the netted condition of the mycelium (*cf.* Fig. 88, p. 158), they can pass around the mycelium tangentially, using numerous lateral hyphae as channels. That they do use such channels has been proved (*vide infra*) by excising the central part of a large haploid mycelium, applying to the periphery of the mycelium remaining a tiny haploid mycelium of opposite sex, and then observing that the large haploid mycelium soon becomes everywhere diploidised all around its periphery. There can be no doubt that the netted condition of a haploid mycelium of *Coprinus lagopus*, and doubtless of other heterothallic Hymenomycetes, is of very great importance for the diploidisation process.

(4) *Mechanical injury.* Vegetative mycelia, like the vegetative parts of all other plants, are liable to injury from various mechanical causes. If a large vegetative mycelium were not a network, any break in one of its thin hyphae would divide it into two parts; but, since it is a network, the breaking of some of its hyphae may still leave it as a unit and capable of acting as such in the formation of a fruit-body. Furthermore, there is always the possibility of

new hyphal fusions being established to repair the damage done by the loss of old ones.

(5) *Social organisation*. As we have seen in previous pages, hyphal fusions enable many mycelia of the same species occupying the same substratum to unite together to form a single compound mycelium which acts as a social unit in the production of fruit-bodies and spores. One only of the components of the compound mycelium may give rise to a fruit-body, in which case all the other components remain sterile but contribute to the reproduction of the species by yielding up their contents to the developing fruit-body.

Sex and Hyphal Fusions in the Hymenomycetes.—With a view to throwing further light on the relation between hyphal fusions and sexual phenomena in the Hymenomycetes, some additional remarks will now be made.

In certain heterothallic species, *e.g.* *Coprinus radians* (= *C. domesticus*) and *C. Rostrupianus*, the spores and the monosporous mycelia are of *two* sexual kinds which may be represented by the symbols (*A*) and (*a*). When a haploid mycelium (*A*) unites with a haploid mycelium (*a*), a diploid mycelium is formed which we may represent by the symbols (*A*)+(*a*), so that, in each of the species under consideration, from the sexual point of view there are three possible kinds of mycelia : (*A*), (*a*), and (*A*)+(*a*). With two sets of mycelia

$$\begin{aligned} &(\textit{A}), (\textit{a}), (\textit{A})+(\textit{a}), \\ &(\textit{A}'), (\textit{a}'), (\textit{A}')+(\textit{a}'), \end{aligned}$$

there are fifteen possible combinations taken two at a time :

$$\begin{aligned} &(\textit{A}) \times (\textit{a}), (\textit{A}) \times (\textit{A})+(\textit{a}), (\textit{a}) \times (\textit{A})+(\textit{a}), \\ &(\textit{A}') \times (\textit{a}'), (\textit{A}') \times (\textit{A}')+(\textit{a}'), (\textit{a}') \times (\textit{A}')+(\textit{a}'), \\ &(\textit{A}) \times (\textit{A}'), (\textit{a}) \times (\textit{a}'), (\textit{A}) \times (\textit{a}'), (\textit{a}) \times (\textit{A}'), \\ &(\textit{A}) \times (\textit{A}')+(\textit{a}'), (\textit{a}) \times (\textit{A}')+(\textit{a}'), (\textit{A}') \times (\textit{A})+(\textit{a}), (\textit{a}') \times (\textit{A})+(\textit{a}), \\ &(\textit{A})+(\textit{a}) \times (\textit{A}')+(\textit{a}'). \end{aligned}$$

From analogous observations made on *C. lagopus* there is every reason to believe that the two mycelia in every one of the fifteen combinations would fuse hyphally with each other, were they given the chance. In general, we may assume that, in a species having two sexual kinds of spores, any one kind of mycelium at one and

the same time can combine with any number of other mycelia with which it may come into contact, whatever may be their sexual condition.

In certain other heterothallic species, *e.g.* *Coprinus lagopus*, *C. niveus*, and *Schizophyllum commune*, the spores and the monosporous mycelia are of four sexual kinds which may be represented by the symbols (AB) , (ab) , (Ab) , (aB) . Of the haploid mycelia, (AB) may unite with (ab) to form a diploid mycelium $(AB)+(ab)$, or (Ab) may unite with (aB) , to form a diploid mycelium $(Ab)+(aB)$; so that, in each of the species under consideration, there are six possible kinds of mycelia: (AB) , (ab) , (Ab) , (aB) , $(AB)+(ab)$, and $(Ab)+(aB)$. With two sets of mycelia:

$$\begin{aligned} & (AB), (ab), (Ab), (aB), (AB)+(ab), (Ab)+(aB), \\ & (A'B'), (a'b'), (A'b'), (a'B'), (A'B')+(a'b'), (A'b')+(a'B'), \end{aligned}$$

there are sixty-six possible combinations taken two at a time. Of these combinations the following are a few examples:

$$\begin{aligned} & (AB) \times (A'B'), (AB) \times (ab), (AB) \times (Ab), (AB) \times (aB), \\ & (AB) \times (AB)+(ab), (AB) \times (A'b')+(a'B'), \\ & (AB)+(ab) \times (A'B')+(a'b'), (AB)+(ab) \times (A'b')+(a'B'). \end{aligned}$$

Twenty-one of the sixty-six combinations were actually made on dung-agar plates, and in every one of them hyphal fusions between the two mycelia were observed (*vide infra*). The union of the two mycelia in the combination $(Ab) \times (Ab)+(aB)$ is illustrated in the next chapter in Fig. 135. From these observations we are justified in concluding that, in *Coprinus lagopus* and similar Hymenomycetes, any two like mycelia or any two unlike mycelia can unite with one another to form a compound mycelium and that, in general, any one kind of mycelium at one and the same time can combine with any number of other mycelia with which it may come into contact, whatever may be their sexual condition.

From the data which have just been set forth it will be seen that, in the heterothallic Hymenomycetes, the formation of hyphal connexions between one mycelium and another has nothing to do with the particular sexual condition of the mycelia concerned. If two mycelia which do not react sexually happen to unite, no association takes place between their nuclei; but, if two mycelia of opposite sex

happen to unite, then through the openings made by the first few hyphal fusions nuclei pass from one mycelium into the other mycelium, conjugate pairs of nuclei are soon established in both mycelia, and the mycelial haplophase quickly changes into the diplophase.

In the mycelium of the homothallic Hymenomycetes, *e.g.* *Coprinus sterquilinus* and *C. stercorearius*, the transition from the haplophase with isolated nuclei to the diplophase with conjugate nuclei is not necessarily preceded by the fusion of two hyphae of the same thallus, for it seems to take place in successive cells of the same hypha by means of some re-arrangement of the nuclei. However, it is possible that very young mycelia of homothallic Hymenomycetes, while still in the haplophase, may unite with one another and that under such circumstances a nucleus of one mycelium may be transferred to the other, with the result that conjugate pairs of nuclei come into existence and the diplophase is initiated ; but this, so far, has not been actually observed. What is certain is that, for the beginning of the sexual act by the formation of conjugate nuclei, heterothallic Hymenomycetes require at least one hyphal fusion between two haploid mycelia of opposite sex, whereas homothallic Hymenomycetes do not require a hyphal fusion of any kind.

The freedom and rapidity with which any two mycelia of the same hymenomycetous species fuse with one another where they come into contact in the same substratum is a prime factor in making possible that social organisation which is so marked a characteristic of the vegetative parts of the Hymenomycetes and of other Higher Fungi.

CHAPTER II

THE EFFECT OF DIPLOID ON HAPLOID MYCELIA IN *COPRINUS LAGOPUS*, AND THE BIOLOGICAL SIGNIFICANCE OF CONJUGATE NUCLEI IN THE HYMENOMYCETES AND OTHER HIGHER FUNGI

Introduction—Definition of the Terms *Diploidisation* and *Diploidise*—*Coprinus lagopus*—Methods—Criteria of Sex—The Four Kinds of Haploid and the Two Kinds of Diploid Mycelia—The Pairing of Haploid and Diploid Mycelia—The Rate of Movement of Nuclei through a Haploid Mycelium which is being diploidised by (1) another Haploid Mycelium or by (2) a Diploid Mycelium—Direction taken by Nuclei in passing through a Haploid Mycelium which is becoming Diploidised—A Sex-factor Analysis of a Diploid Mycelium derived from a Large Haploid Mycelium which has been diploidised by a Small Diploid Inoculum—Observations on the Conversion of Haploid into Diploid Hyphae—The Number of Radial Hyphae of a Large Haploid Mycelium which are converted into Diploid Hyphae through the Action of a Suitable Haploid or Diploid Inoculum—The Frequency of Conjugate Nuclear Division in a Radial Diploid Hypha—Hyphal Fusions between All Possible Kinds of Mycelia—The Diploidisation of a Haploid Mycelium by a Theoretically Incompatible Diploid Mycelium—The Biological Significance of the Diploidisation of a Haploid Mycelium by a Diploid Mycelium—The Biological Significance of Conjugate Nuclei

Introduction.—In the great group of fungi known as the Basidiomycetes, which includes the Hymenomycetes, the Uredineae, the Ustilaginaceae, and the Tilletiaceae, when two haploid mycelia (or their equivalents) of opposite sex unite, two nuclei of opposite sex, each nucleus containing n chromosomes, come together in one cell. However, as a rule, the two nuclei do not unite but remain as a pair of nuclei, $(n)+(n)$, and they divide at one and the same time, so that one pair of nuclei gives rise to two daughter pairs. Such a yoked division of two nuclei is known as *conjugate nuclear division*. In the fungi under discussion, when once a pair of conjugate nuclei has been formed, scores or hundreds of successive conjugate nuclear divisions may follow with the result that, in the end, many thousands

of pairs of conjugate nuclei may come into existence. Finally, such pairs of nuclei come to lie in special cells—in the Hymenomycetes in the basidia, in the Uredineae in the teleutospores, and in the Smut Fungi in the chlamydospores—where they fuse. The fusion nucleus, which contains $2n$ chromosomes, then divides twice (in some Tilletiaceae several times) and, during these divisions, a reduction of the chromosomes accompanied by segregation of sex factors, etc., takes place, so that the four nuclei (in some Tilletiaceae more than four nuclei) which result from the division of the fusion nucleus each contain n chromosomes. Of these nuclei one passes into each basidiospore or its equivalent; and thus each basidiospore is a haploid cell. The basidiospores give rise to haploid mycelia; and, when two such mycelia (or their equivalents) of opposite sex meet and fuse, once more two nuclei of opposite sex become associated as a conjugate pair.

Conjugate nuclei are also present in the Exoascaceae, the Pyrenomycetes, and the Discomycetes, subdivisions of the Ascomycetes, but in the Pyrenomycetes and the Discomycetes they are confined to the ascogonium and to the ascogenous hyphae and young asci of the fruit-bodies, and they are unknown in the vegetative mycelium.

In all animals, in Algae, the Bryophyta, the Pteridophyta, and the Phanerogamia, when two nuclei of opposite sex meet in a cell (usually the egg-cell), they fuse and form a single nucleus containing $2n$ chromosomes, and in all these organisms the phenomenon of conjugate nuclear division is entirely unknown.

In view of the fact that conjugate nuclei are known only in the Basidiomycetes and in certain Ascomycetes, we may ask the question: of what biological advantage, if any, are conjugate nuclei in these two groups of fungi?

In this chapter an attempt will be made to throw light on the significance of conjugate nuclei and conjugate nuclear division, with special reference to the Hymenomycetes.

While reflecting on the problem of the significance of conjugate nuclear division in the Hymenomycetes, it occurred to me that the non-fusion of nuclei of opposite sex in the diploid mycelium of Mushrooms and Toadstools and their association as conjugate pairs is not only an interesting cytological fact, but also may be something

of great physiological importance in that it may permit of a *diploid mycelium diploidising an appropriate haploid mycelium* in dung-balls, wood, or other substrata where haploid and diploid mycelia often intermingle, thus increasing the amount of diploid mycelium produced in the substratum and therefore also increasing the chances that a vigorously fruiting mycelium giving rise to diploid fruit-bodies yielding all the possible sex-groups of spores will be developed rather than a feebly fruiting mycelium giving rise to haploid fruit-bodies yielding but one sex-group of spores. And it further occurred to me that it would be possible to test the value of the theory just propounded in a very simple manner, *i.e.* by pairing a diploid with a haploid mycelium of the same species and observing whether or not the diploid mycelium is able to diploidise the haploid mycelium.

The mating of a diploid with a haploid mycelium in a typical Hymenomycete, namely *Coprinus lagopus*, has, as we shall see, been successfully and repeatedly accomplished. During the course of the investigation, and incidentally thereto, it has been possible, for the first time, to measure the minimum speed with which nuclei pass through a haploid mycelium, both (1) when the haploid is mated with another haploid of opposite sex and (2) when the haploid is mated with a diploid containing nuclei of a sex opposite to those of its own nuclei.

After the experiments recorded in this chapter had been nearly completed, the writer perceived that the organisation of the nuclei in the diploid mycelial hyphae of the Hymenomycetes in conjugate pairs is *essential for the diploidisation of any haploid mycelium whatsoever*, regardless of the kind of mycelium, haploid or diploid, which may initiate the diploidisation process. In the discussion at the end of this chapter, it will be emphasised that the existence of conjugate nuclei in the diploid hyphae of Hymenomycetes is correlated with the *multicellular* and *multinuclear* condition of the haploid mycelia which must be diploidised, and that the advantage of conjugate nuclei $(n)+(n)$ over solitary $(2n)$ nuclei lies in this: that, in any pair of conjugate nuclei, each member of the pair, although constrained to act with its fellow-member in a conjugate nuclear division during cell-division, yet retains its identity, so that it can divide independently of its fellow-member whenever such a division is able to promote the diploidisation of haploid hyphae.

Some of the chief results of the investigations now to be described in detail were communicated to a meeting of the Fifth International Botanical Congress ¹ on August 19, 1930, and were subsequently published in an article in *Nature* ² on November 1, 1930.

Definition of the Terms Diploidisation and Diploidise.—It was in the article in *Nature*, to which reference has just been made, that the writer first introduced the word *diploidisation* to designate in the Hymenomycetes the process by which a haploid cell is converted into a diploid cell, or a haploid mycelium into a diploid mycelium, by the formation of conjugate nuclei within the cell or within the mycelium. Another new word also introduced there was the verb to *diploidise*. A haploid mycelium of one sex may be said to diploidise a haploid mycelium of opposite sex, or two haploid mycelia of opposite sex may be said to diploidise one another.

Coprinus lagopus.—The fungus employed for this investigation was *Coprinus lagopus* (Figs. 107, 108, and 109). This species, as conceived of by me, has been very fully described and illustrated in Volume III of these *Researches*,³ and it has been used as material for experiments on sex under this name by my pupils, Irene Mounce,⁴ W. F. Hanna,⁵ Dorothy Newton,⁶ H. J. Brodie,⁷ and also by myself.⁸

¹ A. H. R. Buller, "The Biological Significance of Conjugate Nuclei in the Basidiomycetes," Fifth International Botanical Congress held at Cambridge, Section for Mycology and Plant Pathology, August 19, 1930.

² A. H. R. Buller, "The Biological Significance of Conjugate Nuclei in *Coprinus lagopus* and other Hymenomycetes," *Nature*, vol. cxxvi, Nov. 1, 1930, pp. 686-689, with seven diagrams.

³ These *Researches*, vol. iii, 1924, pp. 299-327, Figs. 130-147.

⁴ Irene Mounce, "Homothallism and the Production of Fruit-bodies by Monosporous Mycelia in the Genus *Coprinus*," *Trans. Brit. Myc. Soc.*, vol. vii, 1921, pp. 198-217; also "Homothallism and Heterothallism in the Genus *Coprinus*," *ibid.*, 1922, pp. 256-269.

⁵ W. F. Hanna, "The Problem of Sex in *Coprinus lagopus*," *Annals of Botany*, vol. xxxix, 1925, pp. 431-457; also "Sexual Stability in Monosporous Mycelia of *Coprinus lagopus*," *ibid.*, vol. xlii, 1928, pp. 379-389.

⁶ Dorothy Newton, "The Distribution of Spores of Diverse Sex in the Hymenium of *Coprinus lagopus*," *Annals of Botany*, vol. xl, 1926, pp. 891-917.

⁷ H. J. Brodie, "The Oidia of *Coprinus lagopus* and their Relation with Insects." To appear in the *Annals of Botany*, April, 1931.

⁸ A. H. R. Buller, "Experiments on Sex in Mushrooms and Toadstools," *Nature*, Dec. 6, 1924; also A. H. R. Buller and Dorothy Newton, "The Mating Method of Identification of a *Coprinus* growing on Germinating Seeds of Mangel and Sugar-beet," *Annals of Botany*, vol. xli, 1927, pp. 663-670.

There is no doubt that my *C. lagopus* is the *C. lagopus* of Brefeld's *Untersuchungen*.¹



FIG. 107.—*Coprinus lagopus*, a heterothallic Hymenomycete. A pure culture on a horse-dung ball; from a sowing of spores obtained at Birmingham, England. The culture was kept in the dark and brought into the light four days before the photograph was taken. The fruit-bodies are normal in appearance, except for their basal pseudorhiza which was developed while the culture was in the dark. The pilei are black owing to the ripening of the spores on the gills, and they well display their white fibrous scales which are characteristic for the species. Natural size.



FIG. 108.—*Coprinus lagopus*, a heterothallic Hymenomycete. A pure culture on a horse-dung ball; from a sowing of spores obtained at Birmingham, England. The culture was kept entirely in the dark. The stipes have tapering woolly bases, and the pilei, relatively to their width, are longer than pilei grown in the light. The stipes are elongating. The pileus of the fruit-body on the left is black with ripening spores and is about to expand. Natural size.

Mlle Bensaude,² in 1917 and 1918, published her classical papers on the cytology and sexual processes of a fungus which she called

¹ Cf. these *Researches*, vol. iii, 1924, pp. 299–327, Figs. 130–147, and O. Brefeld, *Untersuchungen über Pilze*, Leipzig, Heft III, 1877, pp. 98–108, Taf. VI, Figs. 1–7, and Taf. VII, Figs. 1–5.

² Mathilde Bensaude, “Sur la Sexualité chez les Champignons Basidiomycètes,” *Comptes rendus des Séances de l’Académie des Sciences*, T. CLXV, 1917, pp. 286–289; also *Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes*, Nemours, 1918, pp. 1–156.

Coprinus fimetarius. In 1920, when work on sex in the Hymeno-



FIG. 109.—*Coprinus lagopus*, a heterothallic Hymenomycete. Two fruit-bodies grown under normal conditions of daylight. The smaller fruit-body came up spontaneously on unsterilised horse dung in the laboratory at Winnipeg, while the large one developed in a pure culture (made with spores of Winnipeg origin) on horse dung. In both fruit-bodies, the pilei are grey, owing to the black spores upon the gills. The left-hand one, which is beginning to expand, has not yet begun to shed spores. The right-hand one has already shed a great many spores, some of which have settled upon and have blackened the middle part of the stipe. Natural size.

mycetes was begun at Winnipeg, I was uncertain whether or not

my *C. lagopus* was identical with Mlle Bensaude's *C. fimetarius*. She had found that the haploid mycelia of her fungus refused to fruit, whereas with my fungus I had observed that haploid mycelia fruit, although poorly. Hanna¹ managed to obtain ten successive haploid generations of my fungus, each generation originating from a single spore and terminating in the production of haploid fruit-bodies. Three years ago I had the pleasure of meeting Mlle Bensaude at Kew and there, with the help of the illustrations in my *Researches*,² she and I compared the two fungi. She was convinced that her *C. fimetarius* and my *C. lagopus* are identical species, and she informed me that, subsequently to publishing her papers, she had observed fruit-bodies in one of her haploid cultures. Thus, what seemed to be an important difference between her fungus and my own can no longer be said to exist.

The *Coprinus lagopus* of myself and my pupils may be accepted as identical not only with the *C. fimetarius* of Mlle Bensaude but with the *C. fimetarius* of Kniep and his pupils, of Brunswik,³ of Oort,⁴ and of others, all of whom have made experiments on sex with this fungus.

Brunswik⁵ states that there is a fungus which grows in woods, etc., which is the true traditional *C. lagopus* and that I have wrongly applied the name *C. lagopus* to *C. fimetarius*. He has found that monosporous mycelia of his *C. lagopus* will not mate with the monosporous mycelia of *C. fimetarius* (my *C. lagopus*), and he therefore regards the two species, although very similar morphologically, as distinct from one another. The reasons for my employing the name *C. lagopus* for my species with that name and for discarding the name *C. fimetarius* are set forth in Volume III of these *Researches*⁶ and will not be repeated here.

¹ W. F. Hanna, "Sexual Stability in Monosporous Mycelia of *Coprinus lagopus*," *Annals of Botany*, vol. xlii, 1928, pp. 379-389.

² These *Researches*, vol. iii, Figs. 130-147.

³ H. Brunswik, "Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*," *Botanische Abhandlungen* herausgeg. v. Goebel, Heft V, 1924, pp. 1-152.

⁴ A. P. J. Oort, "The Sexuality of *Coprinus fimetarius*," *Proceedings of the Koninklijke Academie van Wetenschappen te Amsterdam*, vol. xxxii, 1929, pp. 1-6.

⁵ H. Brunswik, *loc. cit.*, pp. 94-95.

⁶ These *Researches*, vol. iii, 1924, pp. 302-303.

Methods.—A spore-deposit of a fruit-body of *Coprinus lagopus* which came up spontaneously in the laboratory on unsterilised

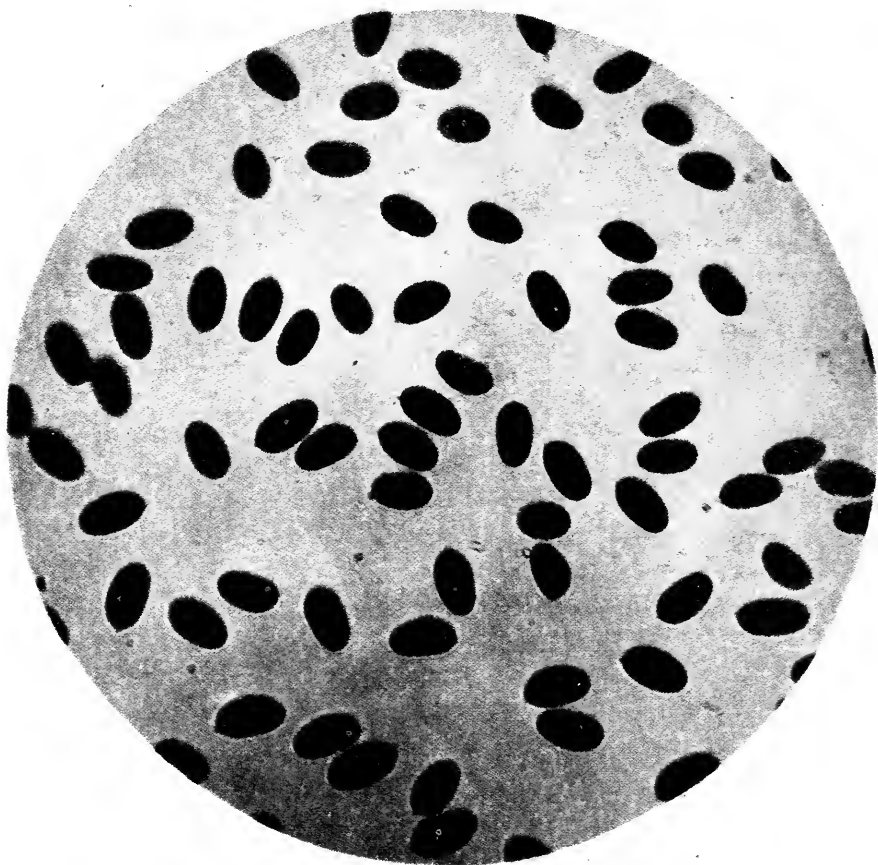


FIG. 110.—*Coprinus lagopus*, a heterothallic Hymenomycete. Photomicrograph of part of a spore-deposit from a small fruit-body which came up spontaneously on unsterilised horse dung at Winnipeg; spores immersed in water. The spores, morphologically, are all alike; but, sexually, as may be determined by mating the mycelia which they produce, they are divisible into four distinct groups to which have been assigned the symbols (*AB*), (*ab*), (*Ab*), and (*aB*). Magnification, 650.

horse dung was collected on a clean sterilised glass slide (*cf.* Fig. 110). A number of spores were then isolated and sown by the dry-needle method¹ at intervals in a Petri dish containing cleared sterilised

¹ W. F. Hanna, "The Dry-Needle Method of making Monosporous Cultures of Hymenomycetes," *Annals of Botany*, vol. xxxviii, 1924, pp. 791-795.

dung-agar. Before the spores were sown, the places where they were to be deposited were marked by means of small circles made with a blue wax pencil on the glass surface of the bottom of the plate. About 90 per cent. of the spores germinated.

When the spores had been in the dung-agar about 24 hours or longer and had produced germ-tubes or young mycelia, they were

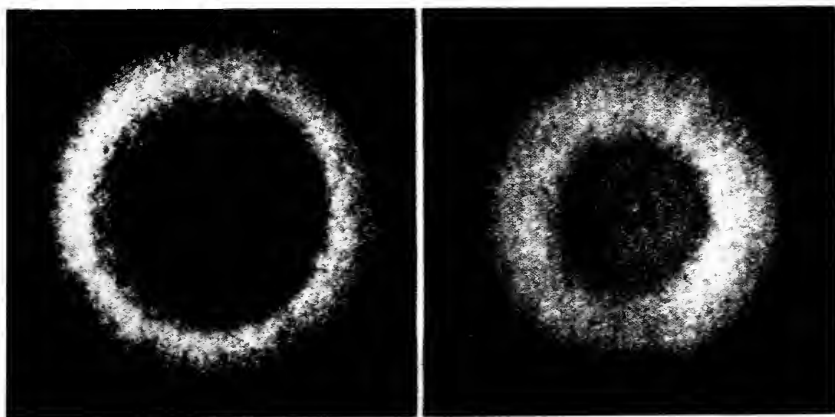


FIG. 111.—*Coprinus lagopus*, a heterothallic Hymenomycete. Two haploid mycelia, each derived from a single basidiospore and growing on cleared dung-agar in the middle of a large Petri dish; photographed against a black background. The one on the left was No. 1 of Table I and had the sexual constitution (*Ab*), and the one on the right was No. 9 of Table I and had the sexual constitution (*aB*). The darkness of the central part of each mycelium is due to the surface hyphae there having become largely or wholly immersed in water excreted by the very numerous oidiophores (*cf.* Fig. 116). More water has been excreted, and probably more oidia have been produced, in the left mycelium than in the right. Photograph taken seven days and seven hours after the inoculation of each of the plates with a tiny mass of aerial hyphae taken from mycelia growing on other plates. The radial rate of growth of the left mycelium (*Ab*) was 0.14 mm. per hour, and that of the right mycelium (*aB*) was 0.13 mm. per hour. Natural size.

transferred with a piece of the dung-agar in which they were situated by means of a flattened nicrome wire loop one by one to separate Petri dishes containing dung-agar. There these monosporous mycelia grew well; and, after an interval of about four days, they were ready for use in mating experiments (*cf.* Fig. 111). When growing, they were kept at room temperature in the light in stacks under a bell-jar.

The dung-agar used as a culture medium was prepared as follows: About 200 grams of fresh horse dung were stirred up in a granite

pan with 1000 cc. water. The mixture was boiled for fifteen minutes



FIG. 112.—*Coprinus lagopus*. Hyphae at the periphery of a large *haploid* mycelium like those shown in Fig. 111 (actually No. 2 of Table I), drawn with the *camera-lucida*. The mycelium was grown on cleared dung-agar, and only the hyphae growing at the surface of the medium have been drawn; lateral hyphae which dipped down into the substratum are shown cut off by a line; the cross-walls (for optical reasons) could not be seen and those shown have been added semi-diagrammatically. The *haploid* nature of the mycelium is indicated by the simple septa, the absence of clamp-connexions, and the wide-angled mode of branching of the leading radial hyphae. The hyphae are as yet all too young to have developed oidiophores and oidia, but these would begin to appear on the older parts of the mycelium here shown about 24 hours later. It will be seen that the lateral branches are more or less tangentially disposed and are effecting anastomoses between one another and the radial hyphae, thus converting the mycelium into a three-dimensional network: already seven hyphal fusions, *ff*, have been made and many more would be made in a short time. Thus, paths which may be used by nuclei of opposite sex when travelling through the mycelium and diploidising it are being rapidly constructed. At *aaa* three lateral hyphae, in the course of their growth at the surface of the dung-agar, have each met with a radial hypha, have thereby been deflected from their original course for a time, and then have suddenly resumed their old direction of growth. This curious phenomenon is not infrequently to be observed in cultures of the kind here described. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 88.

and then filtered through cheese-cloth. Water equivalent to that lost through boiling and filtering was then added (so that the volume

of the decoction was again brought up to 1000 cc.), agar at the rate of 1·2 per cent. was stirred in, and the whole boiled again for twenty minutes or so until all the agar had melted. Water equivalent to that lost in the second boiling was now added and then the medium was filtered through cotton wool into a large beaker and cooled to 60° C. To clear the medium, two eggs were employed. The whites were separated from the yokes, slightly beaten, set in a pan with 50 cc. water, and stirred up with a spoon. The beaten whites,

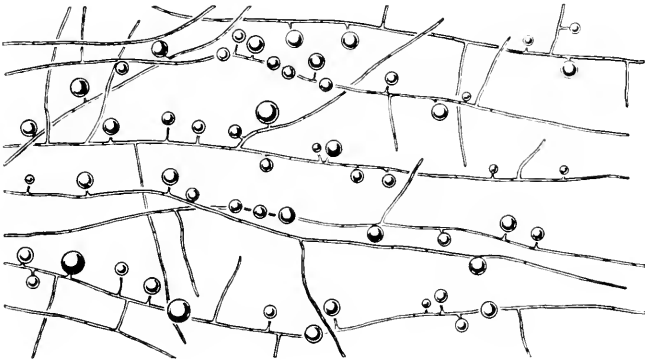


FIG. 113.—*Coprinus lagopus*. A haploid mycelium which has been growing at the surface of dung-agar in a Petri dish for 48 hours. Note the wide-angled mode of branching and the presence of numerous oidial fructifications. Each of these fructifications consists of an oidiophore (projecting away from the substratum into the air) crowned by a drop of fluid enclosing numerous oidia. Drawn, under the direction of the author, by H. J. Brodie. Magnification, 87.

along with their crushed shells, were then added to the dung decoction while this was at 60° C. ; and the whole was stirred very slightly to prevent the whites from completely settling at the bottom of the beaker. The beaker was then set in an Arnold steriliser and steamed for one hour. The clear liquid in the upper part of the beaker was then poured into a filter funnel containing cotton-wool ; and, when it had passed through, the rest of the contents of the beaker containing the coagulum was also poured into the funnel through which its liquid portion slowly passed. The cleared dung-agar was then tubed and sterilised at 15 lb. pressure for one hour in an autoclave.

The Petri dishes used were of two sizes : (1) 10 cm. in diameter,

for cultivating monosporous mycelia and for pairing two mycelia equal in size ; and (2) 15 cm. in diameter, for pairing one very large piece of mycelium (4-6 cm. in diameter) with another very small piece of mycelium.

Criteria of Sex.—In a *haploid* mycelium of *Coprinus lagopus* :

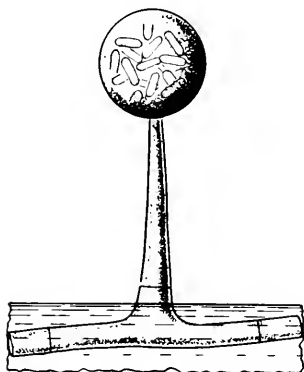


FIG. 114.—*Coprinus lagopus*.

A hypha of a *haploid* mycelium, derived from a single basidiospore and growing in dung-agar, which has given rise to an oidiol fructification. This fructification is aerial, and it consists of an oidiophore crowned with a drop of liquid containing numerous oidia. The oidia, which float freely in the drop, were formed by the breaking into segments of a number of oidiol branches which grew out from the apex of the oidiophore. Drawn, under the direction of the author, by H. J. Brodie. Magnification, 933.

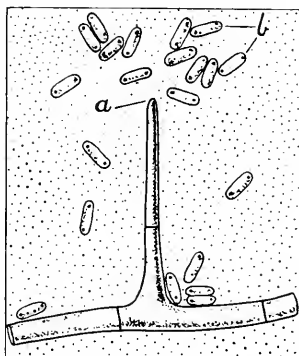


FIG. 115.—*Coprinus lagopus*.

An oidiophore, like that shown in Fig. 114, which has been submerged in the film of water covering the surface of the dung-agar in which the mycelium grew. The oidia, which were in the drop that previously crowned the apex of the oidiophore, are now scattered in the film of water and can be readily seen : *a*, the rounded apex of the oidiophore ; *b*, the oidia. Drawn, under the direction of the author, by H. J. Brodie. Magnification, 933.

(1) the nuclei occur singly in the hyphae ; (2) the septa are simple and devoid of clamp-connexions ; (3) the lateral branches of a leading radial hypha come away at a relatively wide angle, 40° – 90° , varying even past 90° up to 110° ; (4) the mycelium develops numerous aerial oidiophores each crowned by a drop of liquid containing numerous oidia ; (5) the aerial mycelium, as viewed with the naked eye, is less dense, less protuberant, and less conspicuous than that

of a diploid mycelium ; and (6) the aerial hyphae, apart from the oidiophores, excrete relatively few and small drops of liquid along their surface.

In a *diploid* mycelium of *Coprinus lagopus* : (1) the nuclei occur in the hyphae in pairs, and each pair of nuclei divides conjugately ; (2) the septa are provided with clamp-connexions ; (3) the lateral branches of a leading radial hypha come away at a relatively acute angle, 10° – 45° ; (4) the mycelium never produces any oidiophores or oidia ; (5) the aerial mycelium, as viewed with the naked eye, is denser, more protuberant, and more conspicuous than that of a haploid mycelium ; and (6) the aerial hyphae excrete relatively numerous and large drops of liquid at frequent and fairly regular intervals along their surface.¹

Illustrations of a *haploid* mycelium of *Coprinus lagopus* are provided as follows : for the macroscopic appearance, Fig. 111 ; for the wide-angled mode of branching of the leading radial hyphae, for the presence of plain septa and absence of clamp-connexions, and for the formation of hyphal fusions, Fig. 112 ; for oidial fructifications, Figs. 113–116 ; and for solitary nuclei, represented diagrammatically, Figs. 140 and 147, which will be introduced later in a discussion of the diploidisation process (pp. 269 and 279).

Illustrations of a *diploid* mycelium of *Coprinus lagopus* are provided as follows : for the macroscopic appearance, Fig. 117 ; for the narrow-angled mode of branching of the leading radial hyphae,

¹ Richard Harder ("Zur Frage nach der Rolle von Kern und Protoplasma im Zellgeschehen und bei der Übertragung von Eigenschaften," *Zeitschrift für Botanik*, Bd. XIX, 1927, pp. 350–351) observed that in *Pholiota mutabilis*, the rate of growth of a diploid mycelium is about twice as great as that of either of the two haploid mycelia from which it has been derived. By micro-dissection of hyphae he succeeded in obtaining from diploid mycelia uninucleate cells which grew into haploid mycelia. These artificially produced haploid mycelia had a rate of growth of only about half that of the diploid mycelia from which they had come. In *Coprinus lagopus*, in the course of my experiments, I have often noticed that a haploid mycelium, on becoming diploidised, increases slightly its rate of growth (*vide* Figs. 128 and 129). If exact comparative measurements of the rate of growth of a diploid mycelium of *C. lagopus* and of the two haploids from which it has been derived were to be made, it would probably be found that the diploid always grows slightly more rapidly than either of the haploids. Possibly it is a general rule in the Hymenomycetes that diploid mycelia grow faster than their component haploids ; but whether this is so or not needs to be decided by exact investigation.

and for the presence of clamp-connexions, Fig. 118; and for conjugate nuclei and conjugate nuclear division, Figs. 142 and 147.

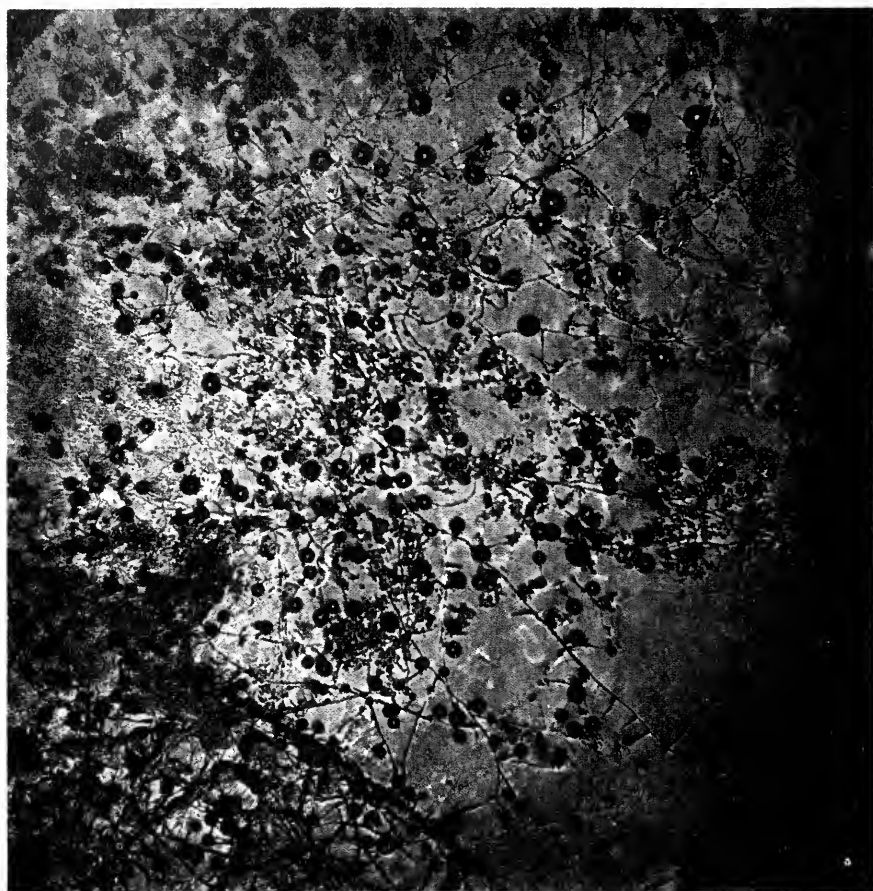


FIG. 116.—*Coprinus lagopus*. Photomicrograph of a *haploid* mycelium, Mr. Brodie's No. 10 (AB), which had been growing for seven days in a hanging drop of malt-agar; to illustrate the production of oidia. The drops which appear as black balls are all filled with oidia, and they are borne on the ends of oidiophores projecting into the air. The very numerous oidia in the film of moisture at the surface of the culture medium arrived at their present position: in part by oïdial drops coming into contact with the film of moisture, in part by being developed from the first on oidiophores lying in the film of moisture, and in part by floating about in the film of moisture. Photomicrograph taken through the cover-glass and the culture medium, under the direction of the author, by H. J. Brodie. Magnification, 100.

In the present investigation no cytological work has been done. In distinguishing a haploid from a diploid mycelium all the five

criteria Nos. 2-6, as listed above, were employed. A haploid mycelium was never regarded as haploid unless its septa proved to

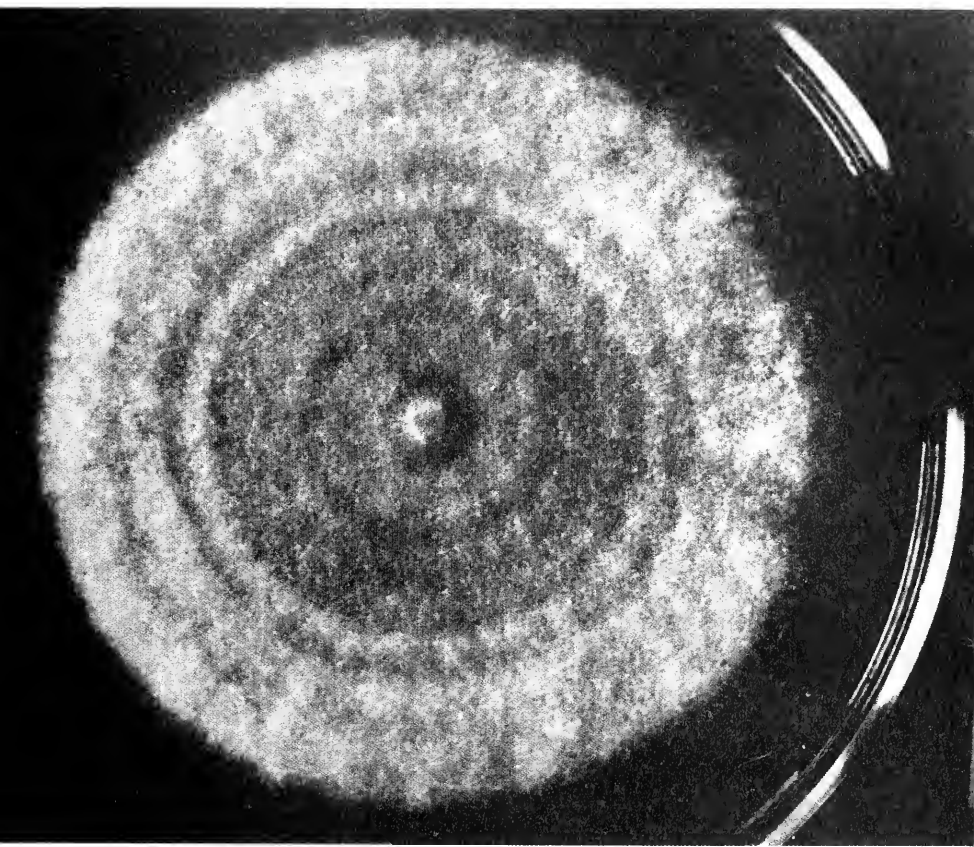


FIG. 117.—*Coprinus lagopus*, a heterothallic Hymenomycete. A diploid mycelium, $(AB)+(ab)$, formed by mating the haploid mycelium No. 2 (AB) of Table I with the haploid mycelium No. 6 (ab) of Table I. The large Petri dish, of which a portion is shown, contained cleared dung-agar. The dung-agar was inoculated at the centre with a small piece of dung-agar (the piece can be seen) in which an $(AB)+(ab)$ mycelium was growing and which had been removed from another dish. The photograph was taken twelve days after inoculation. The radial rate of growth has been about 4.5 mm. per day or approximately 0.19 mm. per hour. The mycelium produced no oidia and was more fluffy at the surface than either of the two haploid mycelia (AB) and (ab) from which it was derived. So far no fruit-body has been formed (for fruit-bodies produced in dung-agar cultures, *vide infra*, Fig. 139). Natural size.

be devoid of clamp-connexions and it exhibited the wide-angled haploid mode of branching; and a diploid mycelium was never

regarded as diploid unless its septa were provided with clamp-connexions and it exhibited the acute-angled diploid mode of branching. When a large haploid mycelium was paired with a small haploid mycelium, the criterion No. 5, which could be applied



FIG. 118.—*Coprinus lagopus*. Hyphae at the periphery of a large *diploid* mycelium like that shown in Fig. 117, drawn with the *camera-lucida*. The mycelium was grown on cleared dung-agar, and only the hyphae growing at the surface of the culture medium have been represented. A few lateral hyphae which dipped down into the medium are shown cut off by a line. The diploid nature of the mycelium is indicated by the clamp-connexions at the septa and by the narrow-angled mode of branching of the leading radial hyphae. The mycelium, as it grew older, would never produce oidia. Cf. the *haploid* mycelium shown in Fig. 112. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 88.

without using the microscope, served as a first indication whether or not the large haploid mycelium was being converted into a diploid mycelium. If the aerial hyphae of the large haploid mycelium became increasingly fluffy around its margin spreading away from the small haploid mycelium, then the conversion of the large mycelium from the haploid to the diploid phase was taking place; but, if no change of this kind could be observed, then the large mycelium was not being converted from the haploid to the

diploid phase. Microscopical examination of the mycelium for presence or absence of clamp-connexions and for the mode of branching constantly confirmed criterion No. 5.

When two paired mycelia growing on dung-agar in a Petri dish were being examined to determine the mode of branching and the presence or absence of clamp-connexions, the Petri dish was usually turned upside down on the stage of the microscope and the mycelium was viewed with the low-power objective. Thus, the dish was not opened, with the result that the mycelia were not subjected to changed conditions of transpiration and, at the same time, the risk of contamination of the medium was reduced to a minimum. Only occasionally, for special or very critical observations, or when the mycelia were not further required, the Petri dish was opened and the mycelia were examined from above with the high-power objective.

The Four Kinds of Haploid and the Two Kinds of Diploid Mycelia.—As determined by mating experiments,¹ the monosporous mycelia (each derived from a single basidiospore) of *Coprinus lagopus* fall into four groups. If it be assumed that each mycelium bears one of each of two pairs of sex factors *Aa* and *Bb*, then the four groups of mycelia and the four groups of spores from which they have been derived may be represented by the symbols: (*AB*), (*ab*), (*Ab*), and (*aB*).

Successful mating of the haploid mycelia of *Coprinus lagopus* with the production of a diploid mycelium (bearing clamp-connexions) is normally possible only in the combinations (*AB*) \times (*ab*) and (*Ab*) \times (*aB*), and therefore the fusion nucleus in every basidium contains all the four sex factors and may be represented by the symbols (*AaBb*).

Since, as a rule, diploid mycelia in *Coprinus lagopus* can be produced only by the combinations (*AB*) \times (*ab*) and (*Ab*) \times (*aB*), but not by the combinations (*AB*) \times (*Ab*), (*AB*) \times (*aB*), (*ab*) \times (*Ab*), or (*ab*) \times (*aB*), we may assume with Kniep² that like factors repel one another while unlike factors attract one another. The mycelia

¹ W. F. Hanna, "The Problem of Sex in *Coprinus lagopus*," *loc. cit.*; also Dorothy E. Newton, "The Distribution of Spores of Diverse Sex, etc." *loc. cit.*

² Hans Kniep, "Die Sexualität der niederen Pflanzen," Jena, 1928, pp. 1-544.

(AB) and (Ab) cannot interact sexually because they have a factor (A) in common.

It is obvious that in *Coprinus lagopus* there are two kinds of diploid mycelia, one derived from the combination $(AB) \times (ab)$ and the other derived from the combination $(Ab) \times (aB)$. These two kinds of diploid mycelia may be represented by the symbols $(AB)+(ab)$ and $(Ab)+(aB)$ respectively.

In what follows, we shall consider the results of pairing a haploid mycelium with a diploid mycelium: (1) when the combinations are $(AB) \times (AB)+(ab)$, $(ab) \times (AB)+(ab)$, $(Ab) \times (Ab)+(aB)$, $(aB) \times (Ab)+(aB)$, *i.e.* when only two kinds of nuclei and all four kinds of sex factors are involved and the combinations seem to be theoretically compatible; and (2) when the combinations are $(AB) \times (Ab)+(aB)$, $(ab) \times (Ab)+(aB)$, $(Ab) \times (AB)+(ab)$, and $(aB) \times (AB)+(ab)$, *i.e.* when three kinds of nuclei and all four kinds of sex factors are involved and the combinations seem to be theoretically incompatible.

The Pairing of Haploid and Diploid Mycelia.—(1) *Preliminary experiments: the two mycelia of equal size.* Mr. H. J. Brodie, working in my laboratory, had in culture a number of monosporous mycelia of *Coprinus lagopus*. Of these his No. 10 and No. 5, which were of opposite sex and to which he had assigned the symbols (AB) and (ab) respectively, were given to me. On mating them, a diploid mycelium $(AB)+(ab)$ was obtained (for the method of mating, *cf.* Fig. 119).

The *haploid* mycelium (ab) and the *diploid* mycelium $(AB)+(ab)$ were then paired on dung-agar contained in a Petri dish. The pairing was accomplished by setting on the dung-agar in the plate, about 1 cm. apart, two small pieces (about 6 square mm.) of dung-agar, one containing the haploid mycelium and the other the diploid.

Two days after the beginning of the experiment, the haploid and the diploid mycelia had come together and hyphal fusions between them were observed.

After another two days, clamp-connexions were observed on all the leading radial hyphae at the periphery of the (ab) mycelium for a distance of 1 cm. on one side of the $(AB)+(ab)$ mycelium.

After one more day (five days from the beginning of the

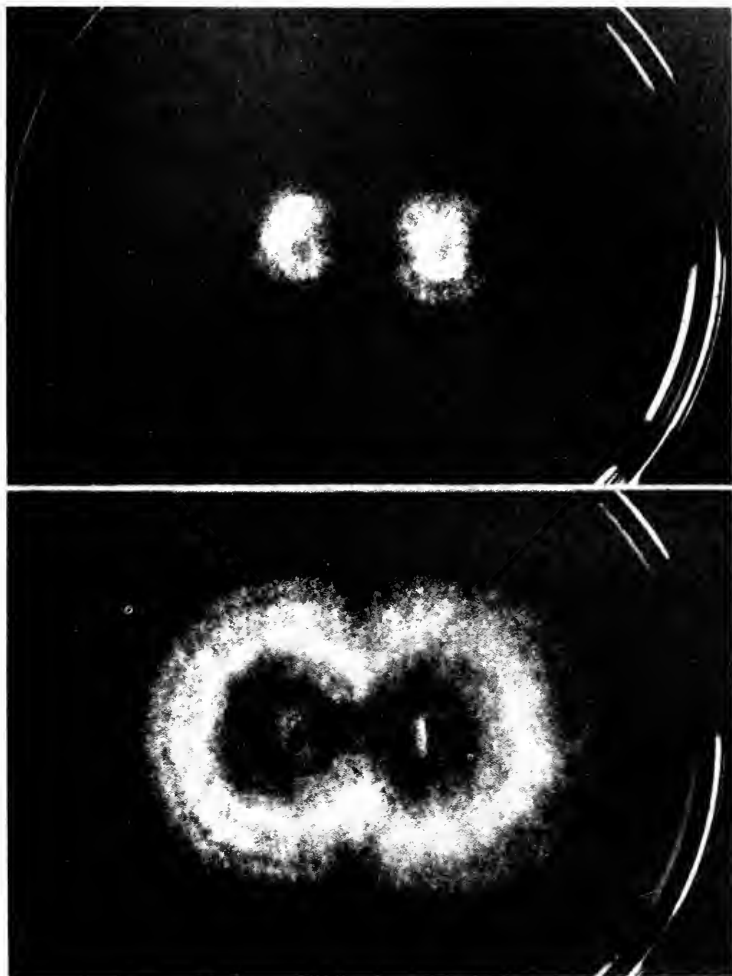


FIG. 119.—*Coprinus lagopus*. Method of mating two mycelia of equal size. A small Petri dish containing cleared dung-agar was inoculated with two pieces of dung-agar, one on the left containing the haploid mycelium (*aB*) and the other on the right containing the haploid mycelium (*Ab*). The upper photograph shows the two mycelia two days after the plate was inoculated; the mycelia have not yet met and both are haploid (cf. Figs. 112 and 116). The lower photograph shows the same two mycelia six days after the plate was inoculated; the two mycelia have met, have fused together hyphally, and have completely diploidised one another, so that all the leading radial hyphae now bear clamp-connexions (cf. Fig. 118) and the production of oidia has ceased. The darkness of the centres of the two original haploid mycelia is due to the surface hyphae there having become largely or wholly immersed in water excreted by the very numerous oidiophores produced before the two mycelia met and diploidised one another (cf. Fig. 111). The two originally haploid mycelia, having now become diploidised, both have the sexual constitution (*Ab*) + (*aB*). Natural size.

experiment), the originally haploid mycelium (*ab*) had become about 2.5 cm. in diameter, and now clamp-connexions were seen to be present on all its peripheral hyphae.

The experiment just described was repeated, with the same result: the haploid mycelium (*ab*) when mated with the diploid mycelium (*AB*)+(*ab*) developed clamp-connexions on all its leading hyphae by the end of five days from the time of pairing the two mycelia.

The results of the two preliminary experiments just described indicated very clearly that a diploid mycelium (*AB*)+(*ab*) is able to diploidise a haploid mycelium (*ab*) when brought into contact with it.

Positive results having been obtained by pairing a haploid with a diploid mycelium of *Coprinus lagopus*, the way was opened for the setting up of further series of experiments which will now be described.

(2) *The haploid mycelium large, the diploid small and set at the periphery of the haploid.* In the preliminary experiments ordinary Petri dishes, 10 cm. in diameter, were employed, and the two pieces of mycelia used for inoculating the dung-agar were of equal size. A change in the mode of experimentation was now made. Wider Petri dishes, 15 cm. in diameter, were employed, and a very large haploid mycelium was paired with a very small diploid mycelium.

Two pieces of the haploid mycelium No. 5 (*ab*) were removed from a stock dung-agar culture and each one was set in the middle of the dung-agar in a freshly-poured large Petri dish; and the two mycelia, one in each dish, were allowed to grow for more than a week. At the end of eight days the diameter of one of the two mycelia was 5.2 cm., and at the end of nine days the diameter of the other was 5.4 cm. When the two (*ab*) mycelia had attained these diameters, a tiny mass of aerial hyphae of the diploid mycelium produced from the combination No. 10 \times No. 5, (*AB*)+(*ab*), scarcely larger than a pin's head, was placed at one spot on the dung-agar in each of the two dishes within 2-3 mm. of the periphery of the large mycelium (*ab*). The relative sizes and positions of the two mycelia resembled (1) at the beginning of the experiment those

of the two mycelia shown in Fig. 124 and (2) one day later those of the two mycelia shown in Fig. 120.

In both of the dishes it was observed that the haploid mycelium (*ab*) became progressively diploidised by the diploid mycelium (*AB*)+(*ab*). The clamp-connexions on the peripheral hyphae of the (*ab*) mycelium were first observed on each side of the (*AB*)+(*ab*) mycelium; and, at the end of four days after pairing, the (*ab*) mycelium was found to have clamp-connexions on the side farthest away from the (*AB*)+(*ab*) mycelium and therefore to have become completely diploidised.

Thus it was proved that a very tiny piece of a diploid mycelium (*AB*)+(*ab*) is able to convert a very large haploid mycelium (*ab*) into a diploid mycelium.

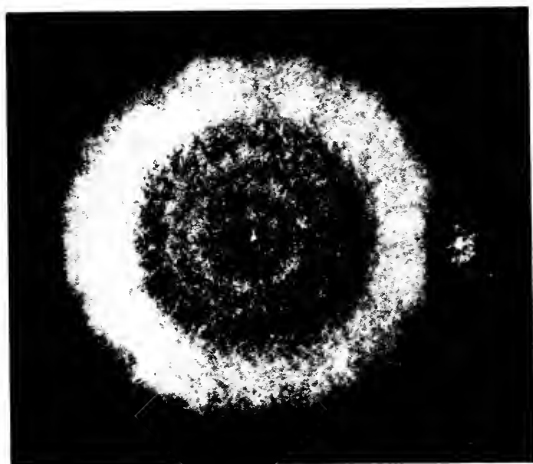


FIG. 120.—*Coprinus lagopus*. Method of mating a large haploid mycelium and a small diploid mycelium. The centre of a large Petri dish containing cleared dung-agar was inoculated with a tiny mass of aerial hyphae of a haploid mycelium (*ab*), No. 6 of Table I. Eight days later, this mycelium had attained a diameter of 4.8 cm. It was then inoculated at its periphery with a tiny mass of aerial hyphae of a diploid mycelium (*AB*)+(*ab*) obtained by mating mycelia No. 6 and No. 2 of Table I. The photograph was taken one day after inoculation. The two mycelia have already come into contact with one another and have fused with one another hyphally. Shortly, the diploid inoculum (*AB*)+(*ab*) will begin to diploidise the large central haploid mycelium (*ab*). Natural size.

(3) *The haploid mycelium large, the diploid small and set in the centre of the haploid.* It seemed possible that the diploidisation of the haploid mycelia in the two series of experiments already described might have been due to nuclei travelling from the diploid mycelium through the peripheral zone of the haploid mycelium and through this zone only. To find out whether or not a diploid mycelium can be effective through the older portions of a large haploid mycelium the experiment now to be described was made.

A piece of the haploid mycelium No. 5 (*ab*) was allowed to grow in a large 15-cm. Petri dish for nine days. At the end of this time, it had attained a diameter of 5.5 cm., whereupon it was inoculated

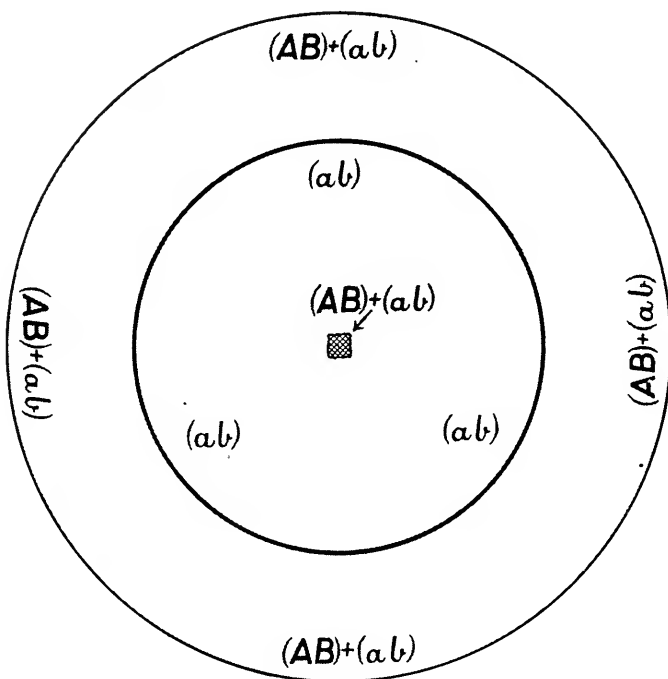


FIG. 121.—*Coprinus lagopus*. The diploidisation of a large haploid mycelium by means of a small diploid mycelium deposited at the centre of the former. A haploid mycelium (*ab*) was allowed to grow on dung-agar in a large Petri dish for nine days, by which time its periphery had extended to the inner darker circle. It was then inoculated at its centre with a small piece of dung-agar (cross-hatched) containing the diploid mycelium (*AB*)+(*ab*). At the end of five days after inoculation, the central (*ab*) mycelium had extended its periphery as far as the outer circle, and all its leading radial hyphae had become completely diploidised (as shown by clamp-connexions). Thus the central diploid inoculum (*AB*)+(*ab*) had diploidised the large haploid (*ab*) mycelium and had converted it into a diploid mycelium (*AB*)+(*ab*). Natural size.

at its centre (Fig. 121) with a small piece of agar (10 square mm.) containing the diploid mycelium No. 10 \times No. 5, (*AB*)+(*ab*).

At the end of five days after the small diploid mycelium (*AB*)+(*ab*) had been placed on the central part of the large haploid mycelium (*ab*), clamp-connexions were observed all around

the periphery of the large and originally haploid mycelium (ab). The radial distance of the periphery of the originally haploid mycelium (ab) from the diploid inoculum (AB)+(ab) at the end of the experiment was about 4.5 cm. (Fig. 121).

From the result of this experiment it is clear that a diploid mycelium (AB)+(ab) is able to diploidise a haploid mycelium (ab) when it has come in contact with only the oldest part of the haploid mycelium; and from the results of the experiments (1), (2), and (3) we may conclude that a diploid mycelium (AB)+(ab) can diploidise a haploid mycelium (ab) if it comes into contact with the latter at any point whatsoever.

(4) *Action of a diploid mycelium on both the kinds of haploid mycelia from which it was derived.* The experiments described under (1), (2), and (3) have shown that a diploid mycelium (AB)+(ab) is able to diploidise a haploid mycelium (ab). Whether or not such a diploid mycelium can also diploidise the haploid mycelium (AB) was settled in the affirmative by means of another set of experiments which will now be described.

Three haploid mycelia No. 10 (AB) were grown separately on dung-agar in three small Petri dishes. When they had attained a diameter of about 3.3 cm., each of them was inoculated at its periphery with a tiny mass of the aerial hyphae of the diploid mycelium No. 10 \times No. 5, (AB)+(ab).

At the end of five days after the diploid mycelium had been added to the dishes, clamp-connexions were present all the way around each of the three originally haploid mycelia (AB). These three mycelia, therefore, had been diploidised by the diploid mycelium.

Having found that the diploid mycelium (AB)+(ab) can diploidise each of the haploid mycelia (AB) and (ab) *separately*, it seemed of interest to determine whether or not the diploid mycelia can convert both the haploid mycelia *simultaneously* into diploids. Accordingly, on one and the same plate of dung-agar there were set in a row three small pieces of dung-agar, the central piece containing the diploid mycelium (AB)+(ab) and the two outside pieces, each 1 cm. distant from the edge of the central piece, containing respectively the haploid mycelia (AB) and (ab) (cf. Fig. 122).

The three pieces of inocula grew well, and the central diploid mycelium soon came into contact with both of the haploid mycelia.

At the end of three days after the experiment had been set up, the (*AB*) mycelium exhibited clamp-connexions all around its

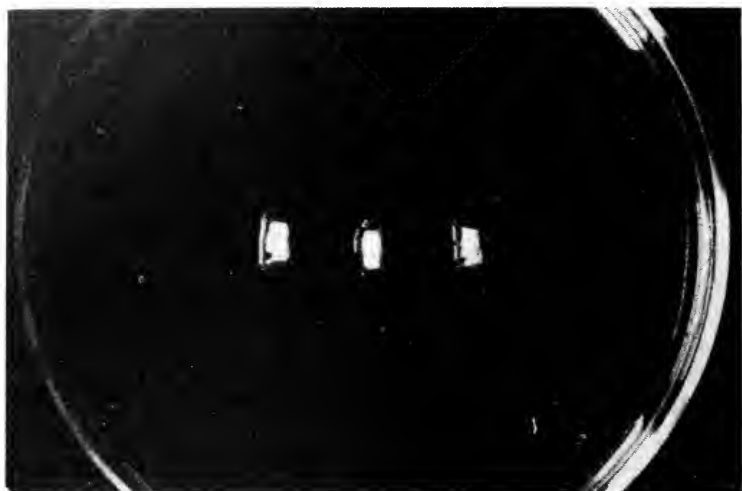


FIG. 122.—*Coprinus lagopus*. The beginning of an experiment in which a diploid mycelium simultaneously diploidised two haploid mycelia like the two from which it was derived. The photograph shows a small Petri dish containing dung-agar, on the surface of which have just been deposited in a row three small pieces of dung-agar containing, from left to right respectively, the following three kinds of mycelia: the haploid mycelium No. 5, (*ab*); the diploid mycelium No. 10 \times No. 5, (*AB*) + (*ab*); and the haploid mycelium No. 10, (*AB*). The mycelia grew out into the dung-agar in the Petri dish and the central diploid mycelium, (*AB*) + (*ab*), soon diploidised both of the lateral haploid mycelia, (*ab*) and (*AB*). Natural size.

periphery and the (*ab*) mycelium clamp-connexions part of the way around its periphery; and, at the end of four days, both of the haploid mycelia had become entirely converted into diploid mycelia (*cf.* Fig. 123). This experiment was repeated and, again, both of the haploid mycelia were rapidly converted into diploid mycelia.

From the results of the experiments described under (1), (2), and (3) combined with the results of the experiments just recorded, we may conclude that a diploid mycelium (*AB*) + (*ab*) is able to convert both of the haploid mycelia (*AB*) and (*ab*) into diploid

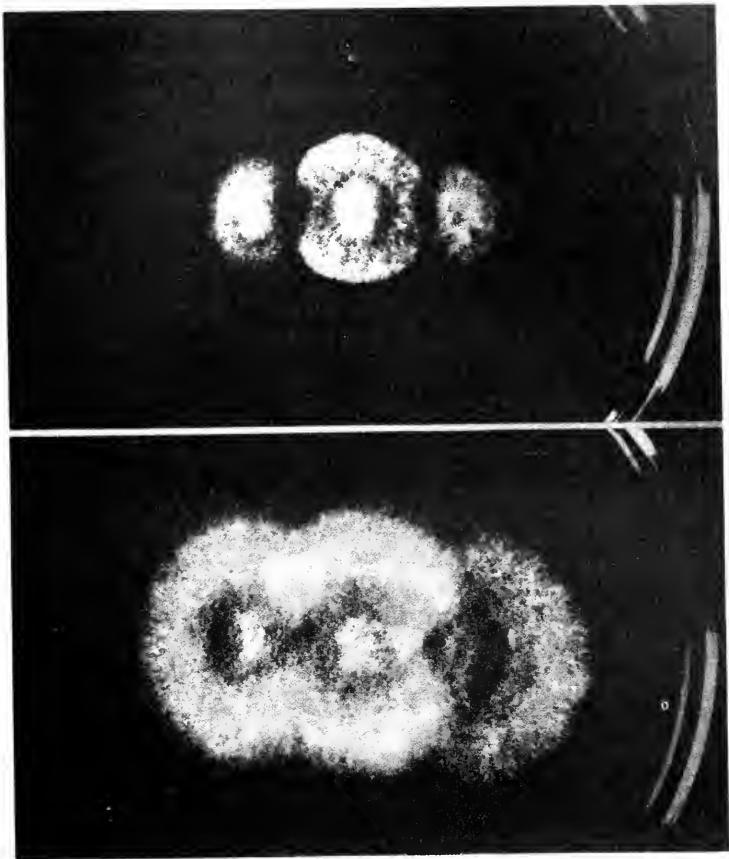


FIG. 123.—*Coprinus lagopus*. Two stages in another experiment in which a diploid mycelium simultaneously diploidised two haploid mycelia like the two from which it was derived. The beginning of the experiment resembled that shown in Fig. 122, except for the fact that the three kinds of mycelia deposited as inocula on the dung-agar plate were, from left to right, as follows: the haploid mycelium No. 1 of Table I, (*Ab*), the diploid mycelium No. 1 + No. 9, (*Ab*) + (*aB*); and the haploid mycelium No. 9 of Table I, (*aB*). Upper photograph, made three days after inoculation: the two lateral haploid mycelia have come into contact with, and have fused hyphally with, the central diploid mycelium. Already the diploidisation of the two haploid mycelia by the central diploid mycelium is almost complete; for clamp-connexions could be observed all around the periphery of the (*Ab*) mycelium and part of the way around the periphery of the (*aB*) mycelium. The central diploid mycelium has grown more vigorously and is fluffier than either of the two haploid mycelia. Lower photograph, made five days after inoculation: clamp-connexions could be seen on all the leading radial hyphae of both the (*Ab*) and the (*aB*) mycelia, thus indicating that the diploidisation of the two lateral haploid mycelia—their conversion into diploid mycelia (*Ab*) + (*aB*)—by the central diploid mycelium (*Ab*) + (*aB*) had been effected completely. The small very white patch on the right side of the central diploid mycelium is due to the presence of a fruit-body rudiment. Natural size.

mycelia, and that it can do this either when it comes into contact with the haploid mycelia one at a time or when it comes into contact with them simultaneously.

(5) *The effect of age on the mating of a diploid with a haploid mycelium.* After the haploid mycelia Nos. 10 and 5, (*AB*) and (*ab*), had been in stock for 5-6 weeks, a series of mating experiments, designed to confirm the experiments already described, was undertaken. In this series of experiments, for the sake of comparison, matings were made not only between a haploid mycelium and a diploid, but also between two haploid mycelia.

Three haploid mycelia (*AB*) and three haploid mycelia (*ab*) were grown on dung-agar, each in a single large Petri dish; and, when they had attained a diameter of 4-6 cm., they were inoculated at their periphery (as in the preceding experiments) as follows:

- (*AB*) with (*AB*)+(*ab*),
- (*AB*) with (*ab*),
- (*AB*) with (*ab*),
- (*ab* with (*AB*)+(*ab*),
- (*ab*) with (*AB*),
- (*ab*) with (*AB*).

In all these six experiments, the large central haploid mycelium, which eventually attained a diameter of 10.0-12.5 cm., became progressively diploidised, and the diploidisation proceeded along its periphery away from, and on both sides of, the inoculum; but the rate of change from the haploid to the diploid phase was much slower than had previously been observed and, when growth ceased in the plates, diploidisation of the central mycelia had been completed only in one of the (*ab*) \times (*AB*) matings. In all the other five matings diploidisation had progressed only from one-half to three-quarters the way around the central mycelium. There was no marked difference between the effect of a haploid mycelium on a haploid mycelium and the effect of a diploid mycelium on a haploid mycelium; but other experiments yet to be recorded will throw further light on this point.

Another set of experiments, similar to those just described, was carried out a month later. Four haploid mycelia were inoculated

on one spot at their periphery when they were about 6 cm. in diameter. The inoculations were as follows :

- (AB) with (AB)+(ab),
- (AB) with (ab),
- (ab) with (AB)+(ab),
- (ab) with (AB).

Again diploidisation of the large central haploid mycelium took place in each combination ; and again its progress was relatively slow. When growth of the mycelia on the plates came to an end, diploidisation had just been completed in the two lower matings listed above, but it had passed no more than half way around the central mycelium in the two upper matings listed above.

The slowness and incompleteness with which the central haploid mycelia were diploidised in the experiments just described, as compared with the relative rapidity and the completeness with which diploidisation had been found to take place in similar experiments described under (2) made two weeks earlier than the set of six matings and six weeks earlier than the set of four matings here treated of, strongly suggests that the results of matings are influenced by age, in that older mycelia effect diploidisation or are diploidised more slowly than younger mycelia that have not long been developed from a germinating spore.

The Rate of Movement of Nuclei through a Haploid Mycelium which is being diploidised by (1) another Haploid Mycelium, or by (2) a Diploid Mycelium. (1) *Eight comparative experiments.* The haploid mycelia Nos. 5 and 10 used in the previous experiments, on account of their having become sexually less vigorous through age, were now discarded. A fresh set of spores was germinated and ten new monosporous mycelia were thus obtained. These mycelia were paired with one another in all possible ways, with results embodied in Table I. In this Table a (+) sign indicates that clamp-connexions were formed and a (—) sign that no clamp-connexions were formed. The mycelia which behaved alike have been set together. All the four possible kinds of mycelia were obtained and to them the symbols (AB), (ab), (Ab), and (aB) have been assigned in the usual manner.

TABLE I

Coprinus lagopus. All Possible Pairings of Ten Monosporous Mycelia derived from Ten Spores of a Single Fruit-body

		AB		ab		Ab			aB		
		2	8	3	6	1	4	5	7	9	10
AB	2	—	—	+	+	—	—	—	—	—	—
	8	—	—	+	+	—	—	—	—	—	—
ab	3	+	+	—	—	—	—	—	—	—	—
	6	+	+	—	—	—	—	—	—	—	—
Ab	1	—	—	—	—	—	—	—	—	+	+
	4	—	—	—	—	—	—	—	—	+	+
	5	—	—	—	—	—	—	—	—	+	+
	7	—	—	—	—	—	—	—	—	+	+
aB	9	—	—	—	—	+	+	+	+	—	—
	10	—	—	—	—	+	+	+	+	—	—

Mycelia No. 2 and No. 6 were mated, as also were No. 1 and No. 9; and thus both possible kinds of diploid mycelia, namely, $(AB)+(ab)$ and $(Ab)+(aB)$ were obtained. A photograph of the diploid mycelium $(AB)+(ab)$ is shown in Fig. 117.

Eight large (15-cm.) Petri dishes containing dung-agar were now inoculated with mycelia at their centre: two with (AB) , two with (ab) , two with (Ab) , and two with (aB) . After nine days the diameter of the mycelia was 5.5–6.2 cm. These large haploid mycelia were then inoculated at one point on their periphery by means of a tiny mass (not much larger than a pin's head) of aerial hyphae taken from near the periphery of another haploid or diploid mycelium grown on dung-agar (Fig. 124).

One of the large (AB) mycelia was inoculated with the haploid (ab) , and the other with the diploid $(AB)+(ab)$. Similarly, (ab) was inoculated with (AB) and with $(AB)+(ab)$; (Ab) was inoculated with (aB) and with $(Ab)+(aB)$; and, finally, (aB) was

inoculated with (Ab) and $(Ab)+(aB)$. After inoculation the eight plates were kept under a bell-jar on a laboratory table at room temperature (about 22°C .).

In all of the eight pairings, the large central haploid mycelium became completely diploidised (as shown by the appearance of clamp-connexions) by the tiny haploid or diploid inoculum within

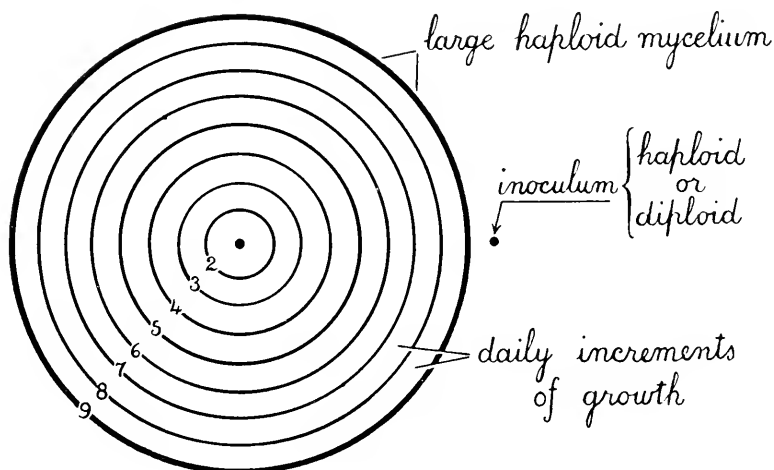


FIG. 124.—*Coprinus lagopus*. Diagram to illustrate the beginning of a mating experiment. A large haploid mycelium has been growing on dung-agar in a Petri dish for nine days. The circles Nos. 2-9 show its boundary at the end of the second to the end of the ninth day. A tiny hyphal mass of another haploid mycelium of opposite sex or of a suitable diploid mycelium, called the *inoculum*, has just been set a little way from the periphery of the large haploid mycelium. Drawn by A. H. R. Buller and Ruth Macrae. Natural size.

four days. Between the time of inoculation and the time of just-completed diploidisation, the large haploid mycelia increased their diameter from 5.5-6.2 cm. to 8.5-9.3 cm.

Photographs of three of the eight pairings :

No. 1, $(AB) \times (ab)$,

No. 2, $(AB) \times (AB)+(ab)$, and

No. 6, $(Ab) \times (Ab)+(aB)$,

taken five days after the large central haploid mycelium was inoculated at its periphery and from one to two-and-a-half days after the large haploid mycelium had become completely diploidised all

around its periphery, are reproduced in Figs. 125, 126, and 127, respectively.

The data for, and the results of, the eight pairings are embodied in Table II. In the eighth column is shown the number of hours taken after inoculation for the complete diploidisation of each of the large central haploid mycelia. In the ninth column is shown the distance in mm. travelled by the nuclei through the large haploid mycelium. This distance was measured from the centre of the inoculum through the centre of the large haploid mycelium to the haploid mycelium's periphery.

By making use of the time and distance data given in columns eight and nine in Table II, the average speed of the nuclei derived from the inoculum when passing through the large central haploid mycelium and diploidising it could be very simply calculated and, as shown in the last two columns of the Table, this speed varied in individual experiments from 0.79 mm. per hour to 1.18 mm. per hour.

It is known from the experimental and cytological work of Lehfeldt¹ that, in *Typhula erythropus*, when two haploid mycelia of opposite sex unite and make a hyphal fusion, a nucleus passes from one of the mycelia into the other and makes its way along the mycelium which it has entered. During its passage, it divides and its progeny divide repeatedly, and the nuclei thus produced by successive nuclear divisions pass along the hyphae and scatter through the mycelium, the hyphal septa breaking down before them to permit their passage. Finally, at a number of points on the mycelium now containing both kinds of nuclei, the nuclei become associated in pairs and clamp-connexions begin to be formed, especially at the ends of the leading hyphae which are rapidly growing in length. It is owing to this division and movement of nuclei which enter a haploid mycelium when it comes into contact with another haploid mycelium of opposite sex that, in the end, any haploid mycelium when mated with another haploid mycelium becomes converted into a diploid mycelium. It is on the assumption that, during its diploidisation, a haploid mycelium of *Coprinus*

¹ W. Lehfeldt, "Über die Entstehung des Paarkernmyzels bei heterothallischen Basidiomyceten," *Hedwigia*, Bd. LXIV, 1923, pp. 13-51.

lagopus behaves essentially in the same manner as a haploid mycelium of *Typhula erythropus* that I have collected the data embodied

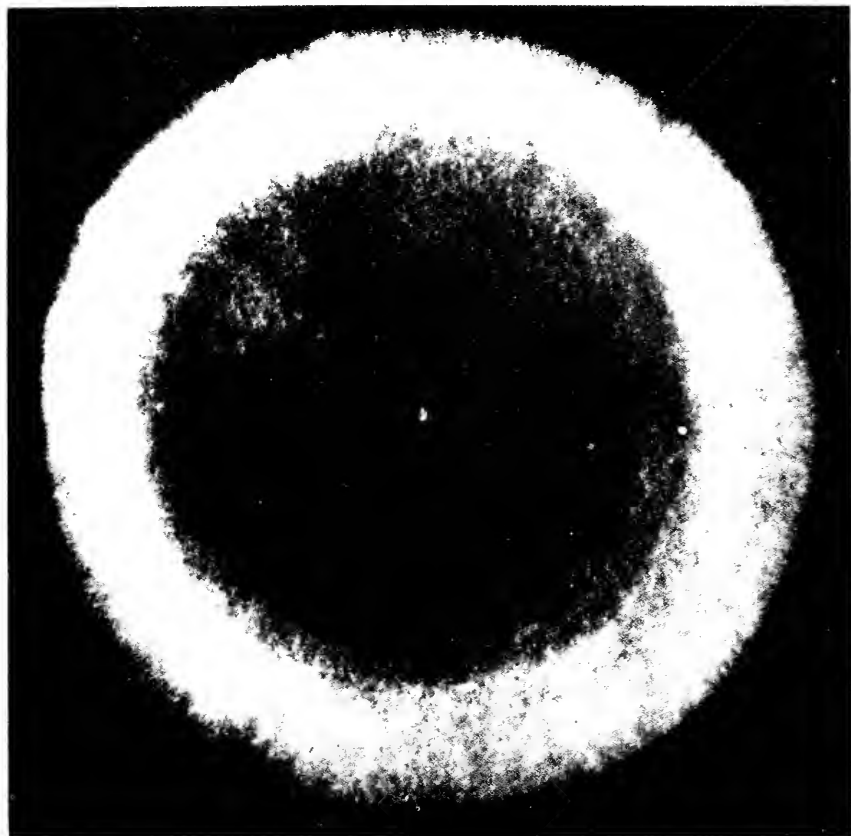


FIG. 125.—*Coprinus lagopus*. The diploidisation of a large haploid mycelium (*AB*) by a small haploid mycelium (*ab*). The large haploid mycelium was allowed to grow on cleared dung-agar until it was 6.0 cm. in diameter. It was then inoculated at its periphery with a tiny hyphal mass of (*ab*) mycelium having the size and position of the white spot which has been marked on the right-hand side of the photograph. At the end of 64 hours after inoculation, the large haploid mycelium had become completely diploidised by the (*ab*) mycelium, as shown by the appearance of clamp-connexions on all its peripheral leading radial hyphae (*vide* Fig. 118). The photograph was taken five days after inoculation and two days after the large haploid mycelium had become completely diploidised. The darkness of the central part of the mycelium is due to the aerial hyphae having become submerged in liquid excreted by oidio-phores when the mycelium was in the haploid state. The hyphae which compose the outer fluffy white ring are diploid and bear no oidiophores. Natural size.

in Table II and have made the calculations of the speed of movement of the nuclei shown there in the last two columns.

(2) *Maximum rate of movement of nuclei.* An attempt was made to determine the maximum speed with which nuclei can pass

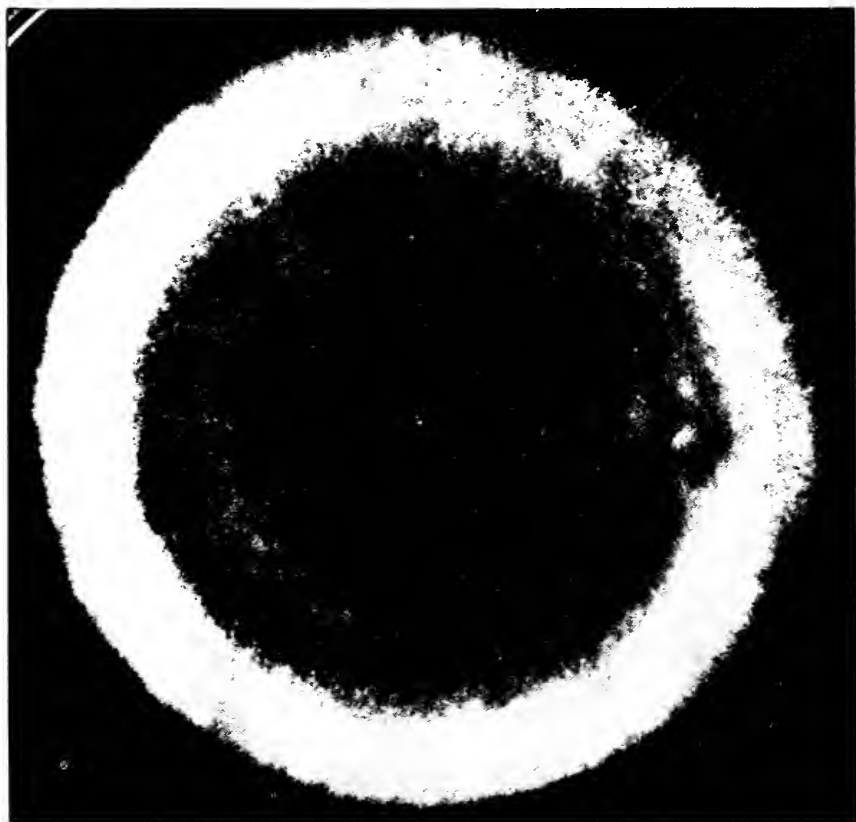


FIG. 126.—*Coprinus lagopus*. The diploidisation of a large haploid mycelium (AB) by a small diploid mycelium (AB)+(ab). The large haploid mycelium was allowed to grow on cleared dung-agar until it was 6.2 cm. in diameter. It was then inoculated at its periphery with a tiny hyphal mass of (AB)+(ab) mycelium which can be observed on the right-hand side of the photograph 1.5 cm. from the periphery. At the end of 74 hours after inoculation, the large haploid mycelium had become completely diploidised by the diploid mycelium, as shown by the appearance of clamp-connexions on all its peripheral leading radial hyphae (*vide* Fig. 118). The photograph was taken five days after inoculation and two days after the large haploid mycelium had become completely diploidised. The darkness of the central part of the mycelium is due to the aerial hyphae having become submerged in liquid excreted by oidiophores when the mycelium was in the haploid state. The hyphae which compose the outer fluffy white ring are diploid and bear no oidiophores. Natural size.

through a haploid mycelium which is being diploidised. Therefore, in the eight experiments just described, the plates were examined

daily to find out how far on each side of the inoculum along the periphery of the large central haploid mycelium clamp-connexions

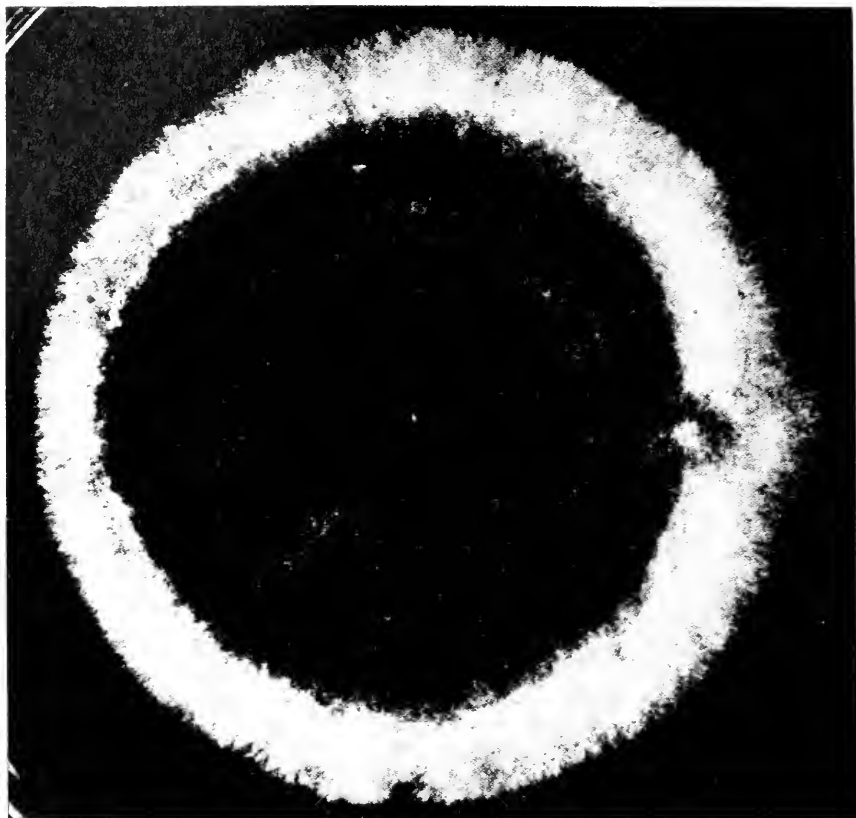


FIG. 127.—*Coprinus lagopus*. The diploidisation of a large haploid mycelium (Ab) by a small diploid mycelium (Ab)+(aB). The large haploid mycelium was allowed to grow on cleared dung-agar until it was 6.2 cm. in diameter. It was then inoculated at its periphery with a tiny hyphal mass of (Ab)+(aB) mycelium which can be observed on the right-hand side of the photograph about 1.5 cm. from the periphery. At the end of 90 hours after inoculation, the large haploid mycelium had become completely diploidised by the diploid mycelium, as shown by the appearance of clamp-connexions on all its peripheral leading radial hyphae. The photograph was taken five days after inoculation and a day and a quarter after the large haploid mycelium had become completely diploidised. The darkness of the central part of the mycelium is due to the aerial hyphae having become submerged in liquid excreted by oidiophores when the mycelium was in the haploid state. The hyphae which compose the outer fluffy white ring are diploid and bear no oidiophores. Natural size.

had been formed, and these points were marked on the under side of the plates.

In all the eight experiments the inocula for the central haploid mycelium and for the excentric haploid or diploid mycelium were tiny masses of surface hyphae (no agar) about the size of a pin's head ; and these inocula in Figs. 124, 128, and 129 are represented by black dots. The limit of growth of each mycelium was marked each day in blue pencil on the under side of the plate and thus a series of concentric circles was obtained. The beginning of a typical experiment, *i.e.* when the second inoculum had just been added to the plate at the periphery of the large central haploid mycelium, is represented in Fig. 124 (p. 215) ; while the end of two experiments, No. 1 and No. 2, is represented in Figs. 128 and 129 respectively.

The greatest speed of nuclear movement (limits of movement shown by the production of clamp-connexions) was found for experiments No. 1 and No. 2 in Table II ; and the data in respect to these experiments will be elucidated with the help of Figs. 128 and 129 respectively.

In both the experiments No. 1 and No. 2 the central haploid mycelium was inoculated at its periphery when nine days old (*cf.* Fig. 124), and the external limit of the central mycelium at that time is indicated for Experiment No. 1 in Fig. 128 and for Experiment No. 2 in Fig. 129 diagrammatically by means of an inner heavier circle (No. 9). The outer heavier circle in Figs. 128 and 129 shows the limit of mycelial growth on the day on which the diploidisation of the large central haploid mycelium had become complete.

(3) *Experiment No. 1 : a haploid mycelium inoculated with a haploid mycelium.* In Experiment No. 1, a large haploid mycelium (*AB*) was inoculated with another haploid mycelium (*ab*) when, as shown in Fig. 128, it was 6.0 cm. in diameter. The time at which the (*ab*) inoculum was added to the plate will be taken as the *zero hour* for the experiment.

It doubtless took several hours for the two haploid mycelia to meet and fuse ; but, by the end of 42 hours after the (*ab*) inoculum had been added to the plate, clamp-connexions were observed at the periphery of the (*AB*) mycelium proceeding away from the (*ab*) inoculum to a distance of 6.0 cm. on one side and 5.3 cm. on

the other side (*vide* the broken lines in Fig. 128). The limits of the progress of clamp-connexion production are shown in Fig. 128 not

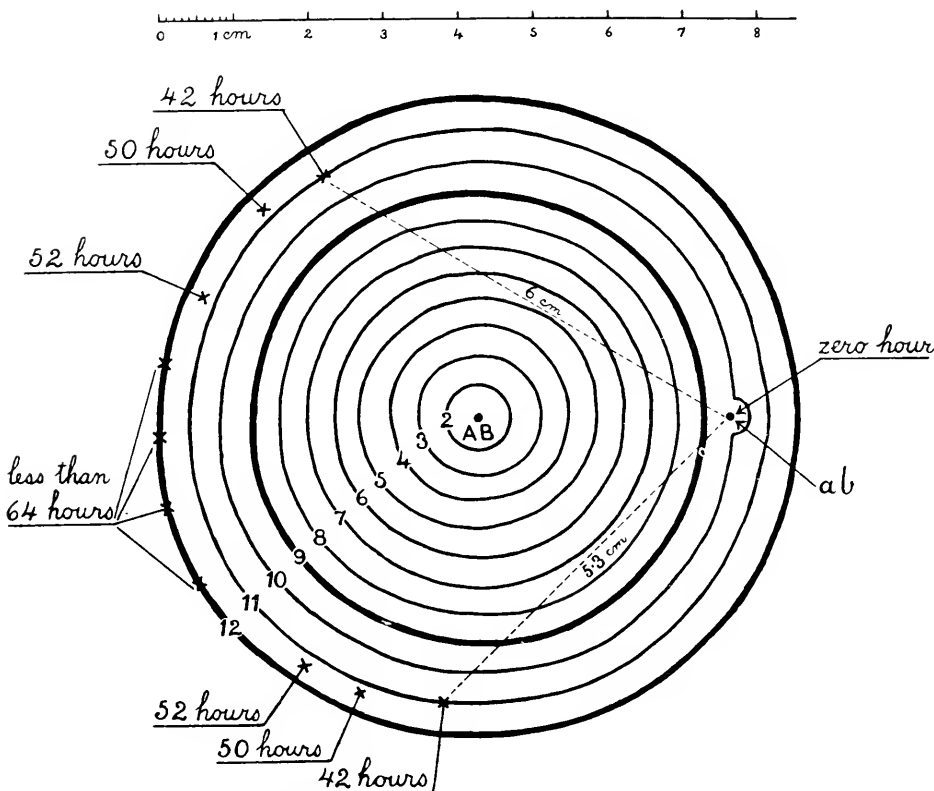


FIG. 128.—*Coprinus lagopus*. The diploidisation of a large haploid mycelium (*AB*) by another haploid mycelium (*ab*) of opposite sex. The circles 2-12, originally drawn in blue pencil on the under side of the Petri dish, show the boundary of the mycelium from the end of the second to the end of the twelfth day. The (*AB*) mycelium was inoculated with a tiny hyphal mass of an (*ab*) mycelium after nine days of growth (periphery shown by the heavier inner circle, No. 9) at the zero hour. The (*ab*) mycelium diploidised the (*AB*) mycelium in the course of three days. The crosses indicate where clamp-connexions were observed after 42, 50, 52, and less than 64 hours, respectively. The (*ab*) nuclei must have travelled (see upper broken line) more than 6 cm. or 60 mm. through the (*AB*) hyphae in about 40 hours, or more than 1.5 mm. per hour. Drawn by A. H. R. Buller and Ruth Macrae. Natural size.

only after 42 hours but also after 50 hours, 52 hours, and 64 hours respectively. At the end of 64 hours (early morning), clamp-connexions were observed all around the part of the central mycelium farthest from the (*ab*) inoculum, and diploidisation of the peripheral

hyphae in this region may have been completed a few hours before the last observation was made.

The greatest rate of movement of the (*ab*) nuclei through the (*AB*) mycelium was, as indicated in Fig. 128, 6.0 cm. in 42 hours, or 1.45 mm. per hour. From the 42 hours we ought to subtract a few hours to allow for the time which elapsed between the zero hour when the inoculum (*ab*) was set on the plate and the time at which the two mycelia (*AB*) and (*ab*) met and fused. Let us subtract two hours only. Then the rate of movement of the (*ab*) nuclei through the (*AB*) mycelium becomes 6.0 cm. in 40 hours or 1.5 mm. per hour.

The rate of radial growth of the central (*AB*) mycelium (average for seven days, rings Nos. 2-9 in Fig. 128 was 0.15 mm. per hour, while the rate of movement of the (*ab*) nuclei through the (*AB*) mycelium was at least 1.5 mm. per hour. A simple calculation based on these data permits us to conclude that the (*ab*) nuclei moved through the hyphae of the central (*AB*) mycelium with a speed ten times as great as the rate of growth in length of the mycelium's peripheral hyphae.

The central haploid mycelium (*AB*), like all other haploid mycelia of *Coprinus lagopus*, consisted of a three-dimensional network of hyphae, the network having been formed by the establishment of thousands of hyphal fusions (*cf.* Figs. 88 and 96, pp. 158 and 170). It is true that the leading hyphae of the mycelium, of which there were many thousands, grew radially away from the centre of the mycelium; but, a little way back from the periphery of the mycelium, all these radial hyphae were connected together laterally by means of their lateral branches.

Since the large central haploid mycelium (*AB*) consisted of a three-dimensional network of hyphae, the (*ab*) nuclei which passed out of the (*ab*) inoculum cannot have progressed through the (*AB*) mycelium in straight-line courses like those indicated by the two broken lines in Fig. 128; rather, they must have taken zigzag courses—moving (let us suppose) first along a radial hypha, then along some more or less tangential bridging hyphae, then along another radial hypha, and so forth. It is therefore not improbable that in 42 hours some of the (*ab*) nuclei travelled nearly twice the

direct distance of 6 cm. shown in Fig. 128. In any case, whatever the actual speed of the movement of the nuclei may have been, we are obliged to regard the calculated average speed of 1.5 mm. per hour for 40 hours as an under-estimate.

Furthermore, it seems very probable that the travelling (*ab*) nuclei did not move along the (*AB*) hyphae at a uniform speed but paused occasionally, possibly for nuclear division. The greatest speed attained by an (*ab*) nucleus in passing along an individual (*AB*) hypha may possibly have been two or three times the calculated average speed of 1.5 mm. per hour, *i.e.* it may have been as much as 3.0–4.5 mm. per hour.

(4) *Experiment No. 2: a haploid mycelium inoculated with a diploid mycelium.* In Experiment No. 2 a large haploid mycelium (*AB*) was inoculated at what we shall again regard as the zero hour with a tiny mass of a diploid mycelium (*AB*)+(*ab*) when, as shown in Fig. 129 by a heavier inner circle, it had been growing for nine days and was 6.2 cm. in diameter. The record for this experiment, embodied in Fig. 129, is similar to that already described for Experiment No. 1.

After 42 hours, it was observed that the central haploid mycelium (*AB*) was becoming diploidised by the diploid mycelium (*AB*)+(*ab*) ; for, already, clamp-connexions were to be seen along the periphery of the former proceeding away from the diploid inoculum to a distance of 3.3 cm. on one side and 4.7 cm. on the other side (*vide* broken lines in Fig. 129).

After 66 hours, diploidisation of the central haploid mycelium had progressed on one side of the diploid inoculum to a distance of 7.0 cm. and on the other side to a distance of 7.7 cm. (*vide* broken lines in Fig. 129).

We may suppose that, when the haploid mycelium (*AB*) and the diploid mycelium (*AB*)+(*ab*) came into contact, hyphal fusions took place between the two mycelia and that one or more (*ab*) nuclei passed out of the diploid mycelium into the haploid mycelium. Some of these nuclei, as the data just recorded indicate, must have travelled through the large central haploid mycelium a distance of 7.7 cm. in 66 hours, *i.e.* at an average speed of 1.17 mm. per hour. If, as before, we subtract two hours from the 66 hours to allow for

time which elapsed between the zero hour when the inoculum (AB)+(ab) was set on the plate and the time at which the large

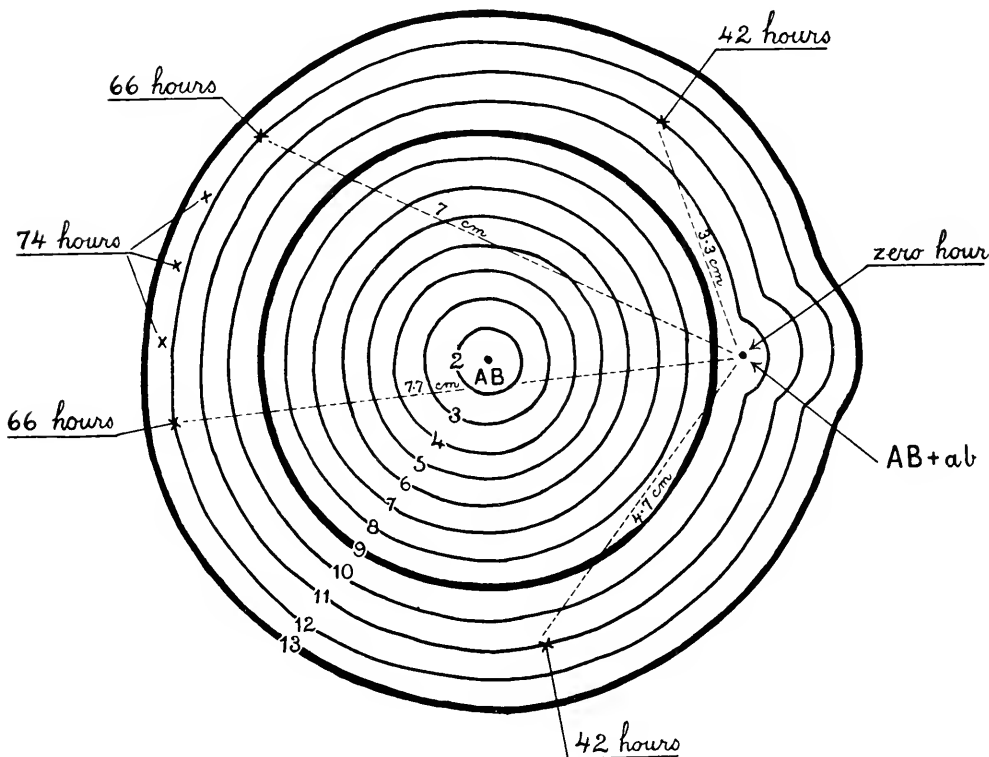
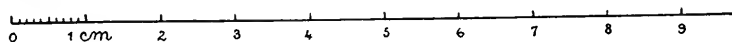


FIG. 129.—*Coprinus lagopus*. The diploidisation of a large haploid mycelium (AB) by a diploid mycelium (AB)+(ab). The circles Nos. 2–13, originally drawn in blue pencil on the under side of the Petri dish, show the boundary of the mycelium from the end of the second to the end of the thirteenth day. The (AB) mycelium was inoculated with a tiny hyphal mass of the (AB)+(ab) mycelium after nine days of growth (periphery shown by the heavier inner circle, No. 9) at the zero hour. The diploid mycelium diploidised the haploid mycelium in a little more than three days. The crosses indicate where clamp-connexions were observed after 42, 66, and 74 hours, respectively. The (ab) nuclei must have travelled (see lower broken line) more than 7.7 cm. or 77 mm. through the (AB) hyphae in about 64 hours, or more than 1.2 mm. per hour. Drawn by A. H. R. Buller and Ruth Macrae. Natural size.

central haploid mycelium and the small excentric diploid mycelium met and fused, the speed of movement of the (ab) nuclei through the (AB) mycelium is increased to 1.2 mm. per hour. Here, again, for

reasons already discussed in connexion with Experiment No. 1, the average rate of movement of the nuclei just calculated, namely, 1.2 mm. per hour, must be regarded as an under-estimate.

(5) *Comparison of the rate of movement of nuclei through (1) a haploid mycelium being diploidised by another haploid mycelium with the rate through (2) a haploid mycelium being diploidised by a diploid mycelium.* As will be seen by reference to Table II, the average rate of movement of the nuclei right across four haploid mycelia being diploidised by *haploid* mycelia (Experiments Nos. 1, 3, 5, and 7) was 0.93 mm. per hour; whereas the comparable average rate right across four haploid mycelia being diploidised by *diploid* mycelia (Experiments Nos. 2, 4, 6, and 8) was 0.89 mm. per hour. The difference between these two average rates scarcely exceeds 4 per cent., and therefore comes within what may be regarded as the limits of experimental error. Hence it appears that the diploidisation of a haploid mycelium can be effected as rapidly by a diploid mycelium as by a haploid mycelium.

In Experiment No. 1, in which a haploid mycelium was inoculated with another haploid mycelium, (*AB*) with (*ab*), the maximum speed of movement of the nuclei, calculated on a direct path for 40 hours, was 1.5 mm. per hour; whereas in Experiment No. 2, the maximum speed of movement of the nuclei, calculated on a direct path for 64 hours, was 1.2 mm. per hour. These were the two maximum speeds observed for any period of time in the eight experiments recorded in Table II. In view of the comparison of average speeds discussed in the preceding paragraph, it seems not unlikely that further work might show that the maximum speed of movement of nuclei through a haploid mycelium is often just as great when a haploid mycelium is being diploidised by a *diploid* mycelium as when a haploid mycelium is being diploidised by a *haploid* mycelium.

(6) *Time taken by a nucleus in travelling a distance equal to its own width.* Another aspect of the movement of nuclei through a haploid mycelium which is becoming diploidised may now be briefly considered. The haploid hyphae of *Coprinus lagopus*, as observed growing on dung-agar in Petri dishes, have a diameter of about 5 μ . In Experiment No. 1, described earlier in this Section

(Fig. 128), if after 42 hours there had been a hypha stretching in a perfectly straight line from the inoculum to the farthest point reached by the (*ab*) nuclei (as shown by the appearance of clamp-connexions), the hypha would have been 6.0 cm. or 60,000 μ long

or $\frac{60,000}{5} = 12,000$ times its own diameter. The width of a nucleus

when passing along a hypha must be less than the width of the hypha, *i.e.* in the case under consideration less than 5 μ . Assuming that the width of each moving nucleus was 4 μ , then a nucleus which took our supposedly straight-hyphal course would have travelled

$\frac{60,000}{4} = 15,000$ times its own width in 40 hours, or 375 times its

own width in one hour, or 6.2 times its own width in one minute, or its own width in about every 10 seconds. But, in reality, owing to the supposed straight-hyphal course not having been available and the actual course having been zig-zag through a three-dimensional network of hyphae, the nuclei under consideration must have travelled upwards of 6.0 cm. in 40 hours. Supposing that in the 40 hours a nucleus actually travelled 10.0 cm., then it must have travelled 25,000 times its own width in 40 hours, or 625 times its own width in one hour, or 10.4 times its own width in one minute, or its own width in about every 6 seconds. If the moving nuclei could have been seen with the microscope, therefore, they would have appeared to move along the hyphae with considerable rapidity.

(7) *The moving nuclei and the septation of the mycelium through which they pass.* The haploid mycelium of *Coprinus lagopus*, like that of other Hymenomycetes, is divided into separate cells by plain septa, but the septa in the species under discussion are arranged at rather irregular intervals along the hyphae. In a hypha of one piece of haploid mycelium there were about 14 septa to one mm. of length. Assuming that, on the average, each mm. of each haploid hypha contains 14 septa, a nucleus passing for 6.0 cm. in a straight course through a haploid mycelium (*cf.* Fig. 128) would be obliged to pass through a hyphal passage-way which initially was provided with 840 septa.

It seems likely that a nucleus, when travelling through a haploid mycelium which it is assisting to diploidise, must, on approaching

a septum, cause the cytoplasm in contact with the septum to break this barrier down or in some way to open a passage through it or round it. Lehfelddt,¹ working with *Typhula erythropus*, actually observed in a mycelium which was becoming diploidised a number of walls which had been dissolved on one side and reduced to "three-quarter" or "half" walls, thus permitting nuclei to pass through them; and, in his Text-fig. 1 *a*, he shows individual nuclei drawn-out and, as it were, in the act of threading themselves through the hole in a partially-dissolved septum. He also states that the lateral wall of a hypha at the side of a partially-dissolved septum was sometimes bulged outwards slightly, as if to increase the width of the septal opening and thus to facilitate the movement of a nucleus when passing through it.

It may well be that, in *Coprinus lagopus*, just as in *Typhula erythropus*, the septa of a haploid mycelium which is being diploidised break down partially or completely before an advancing nucleus of opposite sex; but this supposition remains to be verified or refuted by further observations.

(8) *Concluding remarks on nuclear movement.* The cause of the movement of a nucleus of one sex, say (*ab*), through a haploid mycelium of another sex, say (*AB*), is at present a profound mystery. Obviously, in the supposed particular case, when diploidisation is in progress, the (*ab*) nuclei keep moving away from the diploidised part of the (*AB*) mycelium into the non-diploidised part where, dividing as they go, they finally enter every vigorously-elongating hypha. One must suppose that there is a distinct physiological difference between the already-diploidised and the not-yet-diploidised parts of any mycelium in process of diploidisation or there would be no movement of nuclei from one to the other. Does the difference lie in the cytoplasm only, the nuclei only, or in cytoplasm and nuclei combined; and what is the nature of the difference? To these questions at present we can give no answer.

When, in regarding a large haploid mycelium of *Coprinus lagopus* which is becoming diploidised, we take into consideration its irregularly-netted structure, the thinness of its hyphae, the numerous septa which divide the hyphae into separate cells, and

¹ W. Lehfelddt, *loc. cit.*, p. 14.

the presence in each cell of cytoplasmic and vacuolar contents, the speed with which the invading nuclei advance through the mycelium (in one instance not less than 1.5 mm. per hour and probably 2.0–3.0 mm. per hour) is truly astonishing.

Direction taken by Nuclei in passing through a Haploid Mycelium which is becoming Diploidised.—As we have seen, a tiny pin-head mass of hyphal inoculum of the right kind is sufficient to cause the complete diploidisation of a large haploid mycelium 3–6 cm. in diameter, at least in so far as the peripheral hyphae of the latter are concerned.

One may ask this question: when a large haploid mycelium is being diploidised by a small inoculum, do the nuclei derived from the inoculum travel through the large haploid mycelium in directions which are radial and centrifugal in respect to the inoculum so that some of them pass through the older central part of the mycelium, or do the nuclei travel only tangentially around the mycelium, *i.e.* in a newer and outer zone of the mycelium? In an endeavour to answer this question a number of experiments were made, of which three will now be recorded.

Experiment No. 1. A large haploid mycelium (*ab*), which was 5.2 cm. in diameter, was inoculated at its periphery with a minute fragment of a diploid mycelium (*AB*)+(*ab*), with the result that the large mycelium became diploidised in less than three days.

A few hours after clamp-connexions had been observed to have developed all around the periphery of the large mycelium, there was removed from that portion of the Petri dish where the mycelium was growing a mass of mycelium-covered agar shaped like the hub of a wheel with eight spokes, the hub corresponding to the central part of the mycelium and the ends of the spokes to portions of the periphery of the mycelium. The agar so removed was divided up into one central portion (the hub) and eight radial portions (the spokes), and then these nine pieces of agar were set with intervals between them on dung-agar in another large freshly-poured Petri dish (*cf.* Fig. 130). The mycelium in each of the nine portions of agar removed from the first plate grew out into the fresh dung-agar where it could be readily observed with the microscope. After two days of this renewed growth it was found,

by noting the mode of branching of the hyphae and the presence

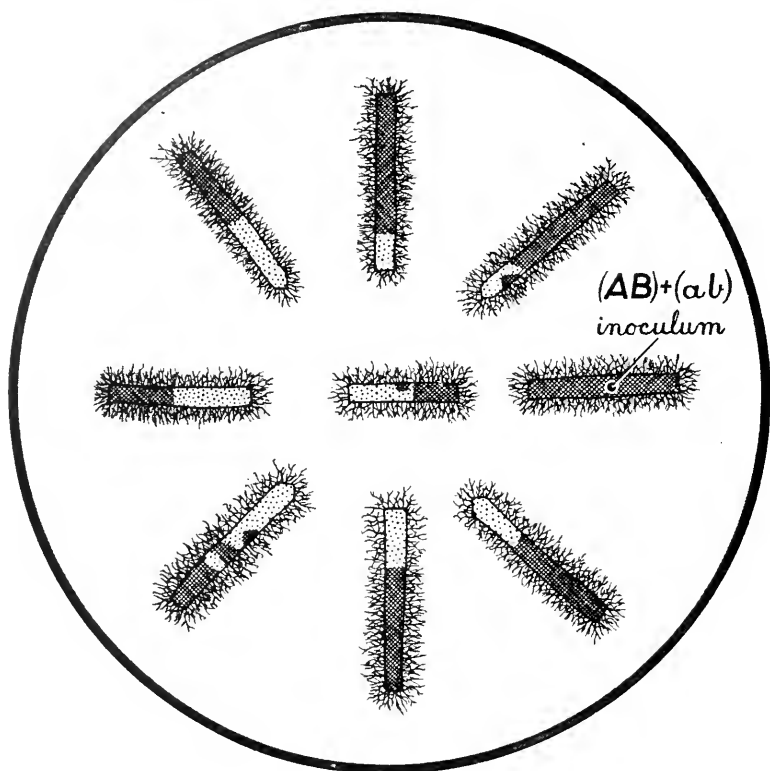


FIG. 130.—*Coprinus lagopus*. Method of determining the haploid or diploid state of various parts of a large haploid mycelium which has just become diploidised by a small diploid inoculum. A haploid mycelium (*ab*) was allowed to grow in a Petri dish on dung-agar until it had attained a diameter of 5.2 cm. It was then inoculated at its periphery with a minute fragment of a diploid mycelium (*AB*)+(*ab*), with the result that it became diploidised in less than three days. A few hours after clamp-connexions had been seen all around its periphery, a piece of the mycelium-covered agar, shaped like the hub of a wheel with eight spokes, was cut out and divided into nine separate pieces which were set down on fresh dung-agar in a Petri dish, at a little distance apart but in their original order, as shown in the illustration. Hyphae grew out from each of the nine pieces into the dung-agar, as indicated, and after two days they were examined with the microscope. The part or parts of each piece which yielded diploid (clamp-connexion-bearing) hyphae have been cross-hatched and the part or parts which yielded haploid hyphae have been dotted. It will be seen that a considerable inner part of the original mycelium must have been still in the haploid condition when diploidisation of all the peripheral hyphae had just been effected. Reduced to two-thirds the natural size.

or absence of clamp-connexions, that: (1) the central piece of

mycelium had developed diploid hyphae on the half nearest to the inoculum and haploid hyphae on the half opposite thereto ; (2) the outer (distal) ends of all the radial pieces of mycelium had developed diploid hyphae ; and (3) the inner ends of all the radial pieces of mycelium had developed haploid hyphae only, excepting that of the piece containing the inoculum which had developed diploid hyphae only and that of one piece next to the piece containing the inoculum which piece had developed both haploid and diploid hyphae (Fig. 130).

From the observations just recorded for Experiment No. 1 we may conclude that, when a large mycelium, through the action of a minute peripheral inoculum, has just become completely diploidised in its peripheral zone (all the hyphae there having clamp-connexions), there are many hyphae in the central part of the mycelium still in the haploid condition but that, toward the centre, proceeding from the inoculum, there are a number of hyphae which have become diploidised.

The observations made in connexion with Experiment No. 1 also seem to indicate that the nuclei derived from a tiny peripheral inoculum travel through a large haploid mycelium more readily in a circle through its younger exterior portions than directly through its older interior portions.

Experiment No. 2. A large haploid mycelium (*AB*) was grown on dung-agar in a large Petri dish until it had attained a diameter of 6·1 cm., and then there was removed from it (together with the agar below) a central portion 3·8 cm. in diameter. Thus there was left in the Petri dish a large hollow ring of mycelium about 1·1 cm. wide. This large mycelial ring was now inoculated at one spot on its outer periphery with a tiny fragment of a haploid mycelium (*ab*), as shown in Fig. 131.

Six days after inoculation, the large hollow ring of mycelium had become diploidised all around its outer margin.

From the results of Experiment No. 2, as just recorded, we may conclude that, when a large haploid mycelium is becoming diploidised by nuclei derived from a small peripheral inoculum, the nuclei can travel *tangentially* all around an outer zone of the mycelium.

It may be added that the experiment just described provides

convincing evidence of the value of the three-dimensional netting of a haploid mycelium in so far as the process of diploidisation is concerned. Had the radial hyphae in the hollow mycelial ring not been all linked up together by numerous bridges formed by their

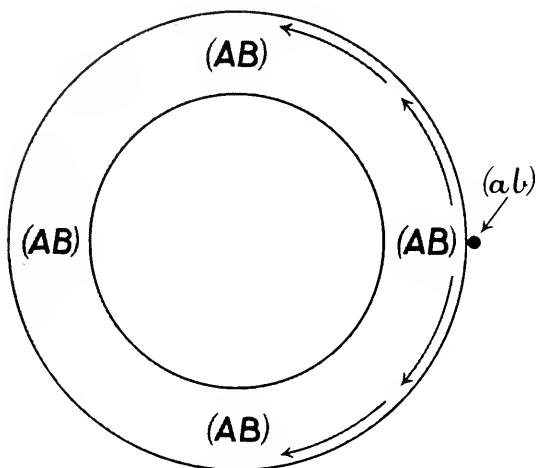


FIG. 131.—*Coprinus lagopus*. The diploidisation of a large haploid mycelium having the form of a hollow ring by means of a small haploid mycelium of opposite sex deposited at its outer periphery. A haploid mycelium (AB) was allowed to grow on dung-agar in a large Petri dish until it had attained a diameter of 6.1 cm. Then there was removed from it (together with the agar below) a central portion 3.8 cm. in diameter. The ring of mycelium left behind in the Petri dish was then inoculated at one spot on its outer periphery with a tiny fragment of a haploid mycelium (ab), as shown in the illustration. Within six days the haploid inoculum (ab) had completely diploidised the large haploid mycelium (AB). The arrows inside the (AB) mycelium on each side of the inoculum indicate the directions in which the (AB) mycelium became progressively diploidised as indicated by the successive appearance of clamp-connexions on all the leading peripheral hyphae. Natural size.

lateral hyphae, the nuclei derived from the inoculum could not possibly have travelled tangentially around the mycelial ring and have brought about the diploidisation of all the tens of thousands of peripheral hyphae on the ring's outer side.

Experiment No. 3.

A haploid mycelium (AB) was grown in a large Petri dish on dung-agar until it had attained a diameter of 6.5 cm. Then there were cut away from it on two opposite sides (together with the agar below) two large and equal outer pieces (g and h in Fig. 132) so as to leave behind in the dish a band of mycelium 6.5 cm. long

and 2.0 cm. wide, the centre of the band being the original centre of the mycelium. Then the band of mycelium (AB) was inoculated at one end with a tiny fragment of a haploid mycelium (ab).

Nine days after inoculation, clamp-connexions began to appear at the end of the mycelial band opposite to that to which the inoculum had been applied and 9.5 cm. distant therefrom (Fig. 132).

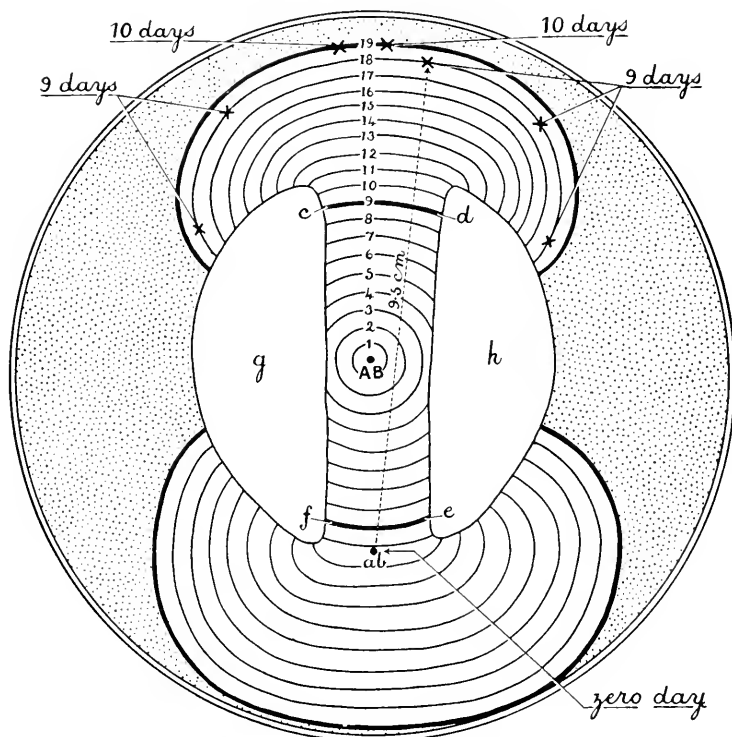


FIG. 132.—*Coprinus lagopus*. Passage of nuclei through the central part of a large haploid mycelium during the diploidisation process. Dung-agar in the large Petri dish was inoculated in the centre with a haploid mycelium (*AB*), and the mycelium grew concentrically for nine days. The rings Nos. 1–9, which were originally made on the base of the Petri-dish with blue pencil, show the daily limits of growth. When the (*AB*) mycelium had attained the size indicated by the inner heavier circle No. 9, two plano-convex lateral pieces of it, *g* and *h*, together with the dung-agar on which they grew, were removed from the Petri dish, so that the (*AB*) mycelium which remained had the configuration *c d e f*. This band of (*AB*) mycelium was then inoculated at one end with a small hyphal inoculum of a haploid mycelium (*ab*). This inoculum, represented by a black dot, was deposited on the culture medium on what may be called the *zero day*. The (*AB*) and (*ab*) mycelia soon fused together. Further growth of the mycelia is indicated by the rings Nos. 10–19 made at the end of each day from the tenth day to the nineteenth day at one end of the central band, and by corresponding rings at the other end. On the ninth day after the inoculum (*ab*) had been placed upon the plate, clamp-connexions were observed at the places shown by crosses on the ring No. 18, while on the tenth day clamp-connexions were observed all around the ring No. 19. Since clamp-connexions are produced during the division of conjugate nuclei, in this case (*AB*) and (*ab*), (*ab*) nuclei must have travelled through the band of (*AB*) mycelium *c d e f* a distance of at least 9.5 cm., as shown by the broken line, in the course of nine days. Drawn by A. H. R. Buller and Ruth Maerao. Two-thirds the natural size.

From the observations just recorded for Experiment No. 3 we may conclude that the nuclei derived from a small peripheral inoculum, when advancing through a large haploid mycelium which is becoming diploidised, can travel through the *middle* and *oldest part* of the mycelium.

The large haploid mycelium used for Experiment No. 3, like all the other large haploid mycelia used for other experiments, had produced a great number of oidiophores and a still greater number of oidia at its surface everywhere except at its extreme edge where its peripheral hyphae were elongating radially. It is therefore clear that nuclei derived from a small peripheral inoculum, when advancing through a haploid mycelium which is becoming diploidised, can move through regions of the mycelium in which oidial production has been very active.

It is certain that, when a large haploid mycelium is becoming diploidised by nuclei derived from a small inoculum, all the actively-growing peripheral hyphae of the mycelium become diploidised, but it may well be that many of the hyphae in the more central and older parts of the mycelium—which have been engaged in the production of oidia, have ceased to grow, and are not likely to form fruit-bodies—may never become diploidised but may remain in the haploid condition until they become exhausted through their contents being drawn to fruit-bodies developing on diploid portions of the mycelium.

Conclusion from Experiments Nos. 1, 2, and 3. The results obtained from the three experiments described above indicate that the nuclei derived from a tiny mycelial inoculum, when advancing through a large haploid mycelium which they are diploidising, may travel through any part of the mycelium, old or young, but that they move more readily through a younger part than through an older part.

A Sex-factor Analysis of a Diploid Mycelium derived from a Large Haploid Mycelium which has been diploidised by a Small Diploid Inoculum.—*Experiment No. 1.* A haploid mycelium (*Ab*), which was No. 1 in Table I (p. 214), was grown on dung-agar in a large Petri dish until it had attained a diameter of 5·2 cm. It was then inoculated at one place on its periphery with a tiny fragment

of a diploid mycelium $(Ab)+(aB)$ which had been produced by mating the mycelia No. 1 and No. 9 of Table I. In the course of six days from the time of inoculation the large haploid mycelium (Ab) became progressively diploidised all around its periphery; and, at the end of that time, all its peripheral radial hyphae displayed the diploid mode of branching and bore clamp-connexions. A piece of mycelium was now removed from the peripheral zone of the large diploidised haploid mycelium at a place in the zone that was farthest from the inoculum (distance from inoculum about 7 cm.), and it was transferred to a wide test-tube (3×1 inches) containing sterilised horse dung. On this culture medium it grew well and, after about ten days, gave rise to fruit-bodies. These were diploid in appearance, and they produced and liberated spores freely, thus functioning in a diploid manner.¹ A spore-deposit was obtained from one of the fruit-bodies and a number of the spores were sown singly in dung-agar, and thus nine monosporous mycelia were obtained.

The nine monosporous mycelia were now paired with one another in all possible ways, with the result that, as shown in Table III, they proved to be divisible into the usual four groups: (AB) , (ab) , (Ab) , and (aB) . The Table III was constructed in the same way as Table I and, here again, a $(+)$ sign indicates that clamp-connexions were produced and a $(-)$ sign that they were not.

We thus find that the diploidised haploid mycelium produced by inoculating a large haploid mycelium (Ab) with a tiny fragment of a diploid mycelium $(Ab)+(aB)$ yielded a fruit-body which liberated all the four possible kinds of spores (AB) , (ab) , (Ab) , and (aB) . From this we are justified in concluding that the fruit-body derived from the diploidised originally-haploid mycelium was truly

¹ Hanna ("Sexual Stability in Monosporous Mycelia of *Coprinus lagopus*," *Annals of Botany*, vol. xlii, 1928, p. 385), working on *Coprinus lagopus* in my laboratory, made the following observations: "As a general rule, diploid fruit-bodies are more vigorous than haploid. Many haploid fruit-bodies do not clongate their stipes, or do not open their pilei, or do not produce any spores; and . . . the most highly developed haploid fruit-bodies produce but relatively few spores, so that their pilei are pale in colour." The pilei of diploid fruit-bodies, when mature, are black in appearance owing to the large number of spores borne on the gills.

diploid, and that the pairs of conjugate nuclei in that mycelium and in the fruit-body produced by it had the constitution $(Ab)+(aB)$.

TABLE III.

Coprinus lagopus. All Possible Pairings of Nine Monosporous Mycelia derived from Nine Spores of a Fruit-body produced on a Large Haploid Mycelium (Ab) after this Mycelium had been diploidised by a Diploid Inoculum $(Ab)+(aB)$.

		AB				ab			Ab			aB	
		3	1	5	8	4	6	9	2	7			
AB	3	—	+	+	+	—	—	—	—	—			
	1	+	—	—	—	—	—	—	—	—			
ab	5	+	—	—	—	—	—	—	—	—			
	8	+	—	—	—	—	—	—	—	—			
Ab	4	—	—	—	—	—	—	—	+	+			
	6	—	—	—	—	—	—	—	+	+			
	9	—	—	—	—	—	—	—	+	+			
aB	2	—	—	—	—	+	+	+	—	—			
	7	—	—	—	—	+	+	+	—	—			

In order to find out whether or not the four groups of mycelia (AB) , (ab) , (Ab) , and (aB) represented in Table III were identical with the four groups of mycelia (AB) , (ab) , (Ab) , and (aB) of the strain of *Coprinus lagopus* from which the diploidised haploid (Ab) and its diploid inoculum $(Ab)+(aB)$ had been derived, the nine mycelia of Table III were paired with Mycelia Nos. 2, 6, 1, and 9 of Table I, i.e. with four testers of the parent strain having the constitution (AB) , (ab) , (Ab) , and (aB) respectively. The results of the pairings are embodied in Table IV.

From the results shown in Table IV, it is evident that the two sets of combinations of sex factors (AB) , (ab) , (Ab) , and (aB) as represented in the parental or original haplonts (monosporous mycelia), on the one hand, and in the filial or new haplonts, on the

other hand, are identical in their sexual behaviour. Hence we may conclude that the diploidisation of the large haploid mycelium (Ab) after coming into contact with the diploid inoculum (Ab)+(aB) was not due to mutation and the introduction of entirely new sex factors such as (a_1B_1) but that, at the beginning of the diploidisation process, one or more (aB) nuclei passed into the large haploid mycelium (Ab) from the diploid mycelium (Ab)+(aB).

TABLE IV.

Coprinus lagopus. Pairings of Four Original Haploid Mycelia with Nine Monosporous Mycelia derived from Nine Spores of a Fruit-body produced on a Large Haploid Mycelium (Ab) after this Mycelium had been diploidised by a Diploid Inoculum (Ab)+(aB).

		New haplonts from (<i>Ab</i>) × (<i>Ab</i>)+(a <i>B</i>)											
		<i>AB</i>				<i>ab</i>				<i>Ab</i>		<i>aB</i>	
		3	1	5	8	4	6	9	2	7			
Original haplonts	<i>AB</i>	2	—	+	+	+	—	—	—	—	—		
	<i>ab</i>	6	+	—	—	—	—	—	—	—	—		
	<i>Ab</i>	1	—	—	—	—	—	—	—	+	+		
	<i>aB</i>	9	—	—	—	—	+	+	+	—	—		

Experiment No. 2. In another experiment, similar to the one just described, a large haploid mycelium (AB), which was No. 2 of Table I, was diploidised by a small diploid inoculum (AB)+(aB), which was derived from the combination No. 2 × No. 6 of Table I.

The results obtained from Experiment No. 2 were similar to, and were interpreted in the same manner as, those obtained from Experiment No. 1. It was concluded that, at the beginning of the diploidisation process, no mutation took place but that one or more (aB) nuclei passed from the diploid inoculum (AB)+(aB) into the haploid mycelium (AB).

Observations on the Conversion of Haploid into Diploid Hyphae.

—A large haploid mycelium (ab), about 5 cm. in diameter, was inoculated at one place on its periphery with a small fragment of

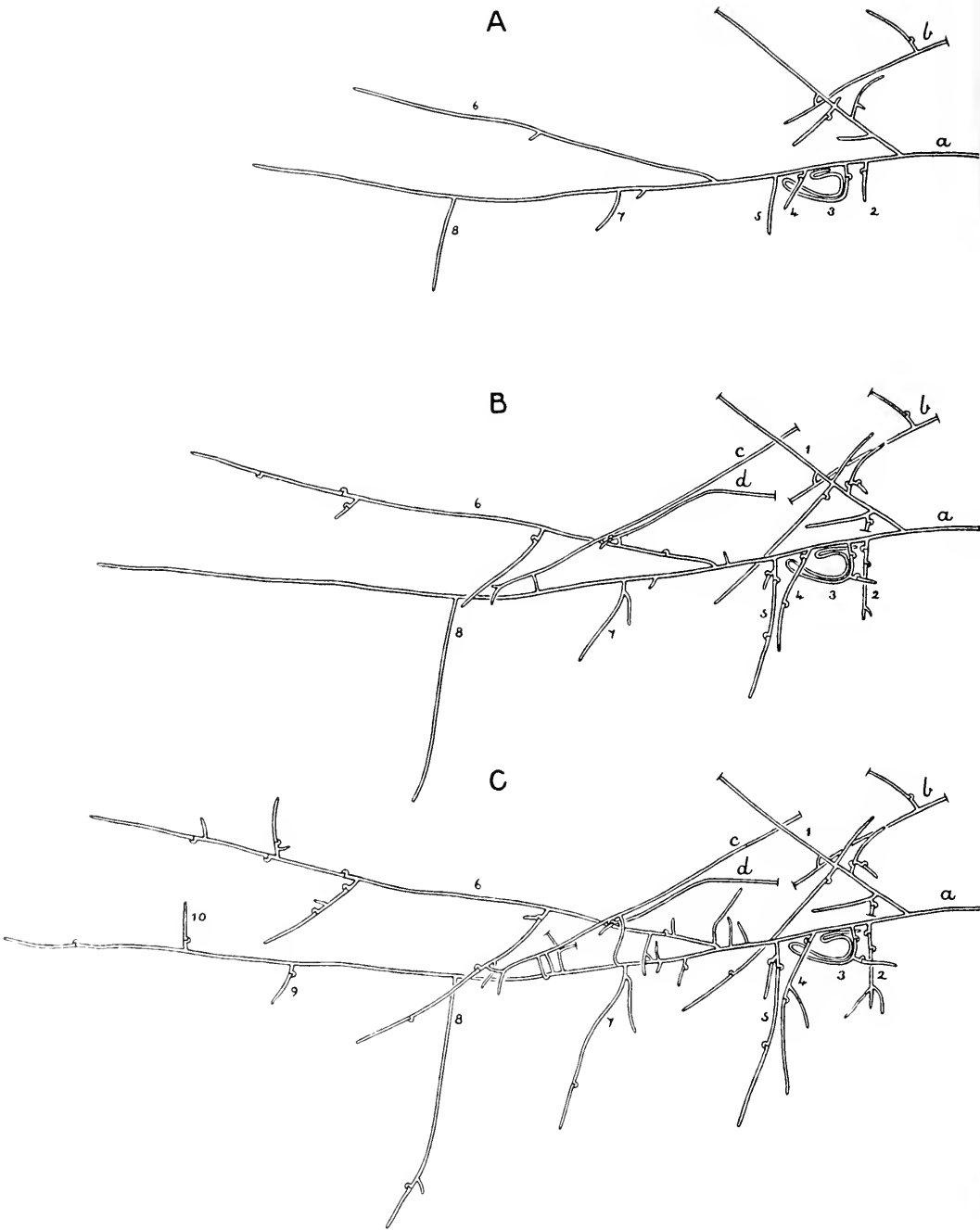


FIG. 133.—*Coprinus lagopus*. Progressive transformation of part of a large haploid mycelium (*ab*) into a diploid mycelium (*AB*)+(*ab*) by nuclei derived from a small inoculum (*AB*). The arrival of the (*AB*) nuclei in the mycelium and the establishment of pairs of conjugate nuclei is indicated by the formation of clamp-connexions.

The large haploid mycelium (*ab*) was grown on dung-agar in a Petri dish; and, when it had attained a diameter of about 5 cm., it was inoculated at its periphery with a tiny hyphal mass of inoculum (*AB*). (Cf. Fig. 124, p. 215.) Three days afterwards diploidisation had advanced most of the way around the periphery of the (*ab*) mycelium proceeding away from the inoculum on both sides (cf. Fig. 128, p. 222). A transition place at the periphery of the (*ab*) mycelium was found where on one side toward the inoculum all the hyphae were already in the diploid phase (provided with clamp-connexions) and on the other side away from the inoculum all the hyphae were in the haploid phase (devoid of clamp-connexions). At this place a leading radial hypha of the (*ab*) mycelium was observed which was being converted from the haploid phase to the diploid phase, and this hypha, marked *a* in the illustration, is shown in three stages of development. It was about 6 cm. distant from the inoculum, it grew in contact with the surface of the dung-agar, and it was drawn with a magnification of 132.

Stage A. The leading radial hypha *a* has eight elongating branches, Nos. 1–8, and one very tiny branch (unnumbered) which grew no further. The branch-hyphae Nos. 2, 3, and 4 have already become diploidised, for each of them has developed a clamp-connexion. The branch-hypha No. 1 has a side-branch with a clamp-connexion. It is likely that an (*AB*) nucleus travelled along the diploid hypha *b* into the branch-hypha No. 1, *via* the short bridging hypha between *b* and No. 1, and thus supplied a conjugate mate for an (*ab*) nucleus in the side-branch which shows the clamp-connexion. The branch-hypha No. 3 is abnormally coiled about itself. The hypha *a* is branched in the wide-angled haploid manner, and it is evident from the disposition of the clamp-connexions on its branches that it is undergoing diploidisation from its older to its younger parts.

Stage B. Drawn 2 hours and 10 minutes after stage A. Three of the side-branches of the branch-hypha No. 1 now bear clamp-connexions, and additional clamp-connexions have developed on branch-hyphae Nos. 2, 3, and 4. Branch-hyphae Nos. 5 and 6 have now become completely diploidised and are developing clamp-connexions in acropetal succession. On No. 6, between *d* and the number 7, a clamp-connexion has developed on the main axis far from the growing point, which is somewhat unusual. The last clamp-connexion to appear on No. 6 was the terminal one. The hyphae *c* and *d* are lateral branches of other leading radial hyphae not shown in the illustration; they have become united, by means of bridging hyphae, with the main axis of *a* and with the branch-hypha No. 6, respectively. Again it is evident that the hypha *a* is undergoing progressive diploidisation from its older to its younger parts.

Stage C. Drawn 4 hours and 10 minutes after Stage A and 2 hours after Stage B. The branch-hyphae Nos. 7 and 8 and also two newly-developed branch-hyphae Nos. 9 and 10 now bear clamp-connexions and therefore have become diploidised, and a clamp-connexion has at last appeared on the main axis of the hypha *a* near its growing point. Thus the hypha *a* with all its ten branches has now become completely diploidised. It was originally part of a haploid mycelium (*ab*); but now, as a result of the entry into it of (*AB*) nuclei which have formed conjugate mates with its own (*ab*) nuclei, it has been transformed into part of a diploid mycelium (*AB*)+(*ab*).

The progressive conversion of the mycelium into a three-dimensional network is evident from a comparison of the drawings A, B, and C. In A there is one hyphal fusion, in B there are three, and in C there are six. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 88.

another haploid mycelium (*AB*) ; and, three days after inoculation, the large mycelium (*ab*) was becoming progressively diploidised around its periphery on both sides of the inoculum. At the periphery of the mycelium (*ab*) a place was found, about 6 cm. from the inoculum, where there was a transition from hyphae producing clamp-connexions regularly and therefore completely diploid to hyphae not yet producing clamp-connexions and therefore completely haploid.

In the transition zone just described particular *haploid hyphae* were watched with the microscope for several hours and their *conversion into diploid hyphae* was actually observed, as will now be recorded in a particular case.

A leading hypha growing radially between diploid and haploid hyphae at the periphery of the (*ab*) mycelium was branched in the wide-angled haploid manner, and there were no clamp-connexions on it or on its four youngest branches (Fig. 133, A, branches Nos. 5, 6, 7, and 8) ; but three of its older branches (Nos. 2, 3, and 4) each bore a single clamp-connexion. It seemed very probable that the hypha in question was being converted from a haploid to a diploid state ; and, therefore, it was kept under continuous observation until, at the end of four hours and ten minutes, the change had been completed. To observe the hypha, the cover of the Petri dish was removed and then the low-power or the high-power of the microscope (magnifications 132 and 500) was applied to it. Between observations the cover was set on the Petri dish to prevent undue loss of moisture from the culture medium. The hypha under observation was growing along the surface of the agar and therefore did not dry up when exposed to the air.

The first state of the haploid hypha in process of being converted into a diploid hypha is shown in Fig. 133, A, where it will be seen that the three lower branches of the hypha *a*, Nos. 2-4, bear clamp-connexions, whereas the four upper branches, Nos. 5-8, have no clamp-connexions whatever. The hypha with its branches was sketched with the low-power of the microscope at 1.30 P.M.

At 3.40 P.M., the hypha *a*, along with its branches, was sketched again (Fig. 133, B) ; and a comparison of the drawings A and B in Fig. 133 shows that, in the intervening two hours and ten

minutes: (1) No. 5, the *fourth* branch from the end of the main hypha, which at 1.30 P.M. had been clamp-less, now bore two clamp-connexions as well as a tiny lateral branch also provided with a clamp-connexion; and (2) No. 6, the *third* branch from the end of the main hypha, which at 1.30 P.M. had also been clamp-less, now bore three clamp-connexions, while its own two short branches each bore a single clamp-connexion. At 3.40 P.M. there was left to be diploidised only the terminal portion of the main hypha *a* and the main hypha's two youngest lateral branches.

After 3.40 P.M. the terminal part of the main hypha *a* not yet diploidised produced two more lateral branches, so that it came to have four branches below its apex, Nos. 7-10, still in the haploid condition. The formation of a clamp-connexion on the main hypha a short distance behind its apex and of clamp-connexions on all the four youngest haploid branches of the main hypha was observed between 3.40 P.M. and 5.45 P.M., by the end of which time, therefore, the whole of the main hypha and its branches had become converted from the haploid to the diploid condition (Fig. 133, C).

A comparison of the drawings A, B, and C in Fig. 133 gives a sufficiently adequate idea of how any leading radial haploid hypha along with its branches, when viewed externally, is converted from the haploid to the diploid state under the influence of nuclei which have come to it from a mycelium of opposite sex.

Similar observations to those just recorded were made on a large haploid mycelium (*AB*) when this mycelium was being converted into a diploid mycelium by a small *diploid* inoculum (*AB*)+(ab). We may therefore conclude that the diploidisation of a haploid mycelium, so far as external changes are concerned, is effected by a suitable diploid mycelium in the same manner as by a suitable haploid mycelium.

The Number of Radial Hyphae of a Large Haploid Mycelium which are converted into Diploid Hyphae through the Action of a Suitable Haploid or Diploid Inoculum.—A small piece of a haploid mycelium, after being set in the middle of a dung-agar plate, grows out radially from its centre; and, when its periphery is examined, the leading radial hyphae can be readily recognised as such.

A large haploid mycelium (*AB*), 6 cm. in diameter (*cf.* Fig. 124,

p. 215), growing on a layer of dung-agar 2-3 mm. thick contained in a 15-cm. Petri dish, was found to have 121 leading radial hyphae included within each mm. of its periphery (average of several counts). The circumference of the mycelium was $60 \times \frac{22}{7} = 188$ mm.

The total number of leading radial hyphae at the periphery of the haploid mycelium was therefore 121×188 or, in round figures, 22,750.

When a 6-cm.-wide haploid mycelium (*AB*) is inoculated at its periphery with a tiny fragment of a haploid mycelium (*ab*) or of a diploid mycelium (*AB*)+(*ab*), all its leading radial hyphae are converted into diploid hyphae bearing clamp-connexions. This conversion, as we have seen from the results of experiments already described, takes about three days, and the haploid mycelium, during this time, increases its diameter to about 8.5 cm. Since the mycelium undergoing diploidisation increases its diameter from 6 cm. to 8.5 cm. before the process is completed, the number of its leading hyphae which are converted from the haploid to the diploid state lies between 22,750 and 32,300. We may therefore assume that, at the periphery of the large haploid mycelium under consideration, the number of leading radial haploid hyphae which receive nuclei from the inoculum is not less than 26,000.

Let us suppose that only one nucleus has passed into the large haploid mycelium from the inoculum. Then, to produce the 26,000 nuclei required to make up the conjugate pairs in the 26,000 end-cells of the 26,000 leading hyphae of the large (*AB*) mycelium, the single (*ab*) nucleus which entered the (*AB*) mycelium would need to undergo subdivision until its progeny consisted of at least 26,000 nuclei. If this subdivision were to be in the form of a geometrical progression in which the original (*ab*) nucleus divided once, its two daughter nuclei once, the resulting four nuclei once, and so forth, a succession of 14-15 divisions would be required to produce the 26,000 (*ab*) nuclei; but, doubtless, the actual series of nuclear divisions is never so regular as that suggested. If some nuclei cease to divide during the diploidisation process, then others must divide more often.

When a large 6-cm.-wide haploid mycelium (*AB*) is becoming diploidised through the action of an inoculum (*ab*) or (*AB*)+(*ab*),

not only do the 26,000 end-cells of the (*AB*) mycelium receive (*ab*) nuclei, but also several of the subterminal cells of each of the leading hyphae, all the younger lateral branches of the leading hyphae, as well as a great number of the hyphae which are not so peripherally situated (interior hyphae). The number of (*ab*) nuclei required to effect diploidisation of our large (*AB*) mycelium is therefore far greater than 26,000 and may well exceed 250,000.

The Frequency of Conjugate Nuclear Division in a Radial Diploid Hypha.—A leading radial hypha in a diploid mycelium of *Coprinus lagopus* growing on dung-agar in a large Petri dish produced clamp-connexions at very regular distances apart. The average distance between two successive clamp-connexions was found to be 0.18 mm. ; so that, in a hypha 1.0 cm. long, there are about 55 clamp-connexions.

A leading radial hypha of a diploid mycelium was kept under observation and was sketched at successive intervals of time during a period of twelve hours ; and thus the number of successive clamp-connexions which it developed was determined. Fourteen clamp-connexions were formed in the twelve hours ; so that, on the average, one new clamp-connexion was formed every fifty minutes. Now it is known from the work of Kniep¹ and Mlle Bensaude² that a conjugate nuclear division takes place whenever a clamp-connexion is formed. We may therefore conclude that, in the diploid hypha that was under investigation, a conjugate nuclear division took place every fifty minutes.

The time required for a single conjugate nuclear division is always a little less than that required for the complete development of a clamp-connexion (*cf.* Fig. 85, p. 155). A clamp-connexion is formed in much less than fifty minutes, certainly in less than thirty minutes. It may well be that a single conjugate nuclear division takes place in less than fifteen minutes.

Hyphal Fusions between All Possible Kinds of Mycelia.—From eight spores two complete sets of haploid mycelia (*AB*), (*ab*), (*Ab*),

¹ Hans Kniep, "Beiträge zur Kenntnis der Hymenomyceten, III," *Zeitschrift f. Botanik*, Bd. VII, 1915, pp. 369-398.

² M. Bensaude, *Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes*, Nemours, 1918, pp. 1-156.

(*ab*) were obtained, and the haploid mycelia of each set were used to make up the two possible kinds of diploid mycelia (*AB*)+(*ab*) and (*Ab*)+(*aB*). Thus, altogether, there were in hand twelve

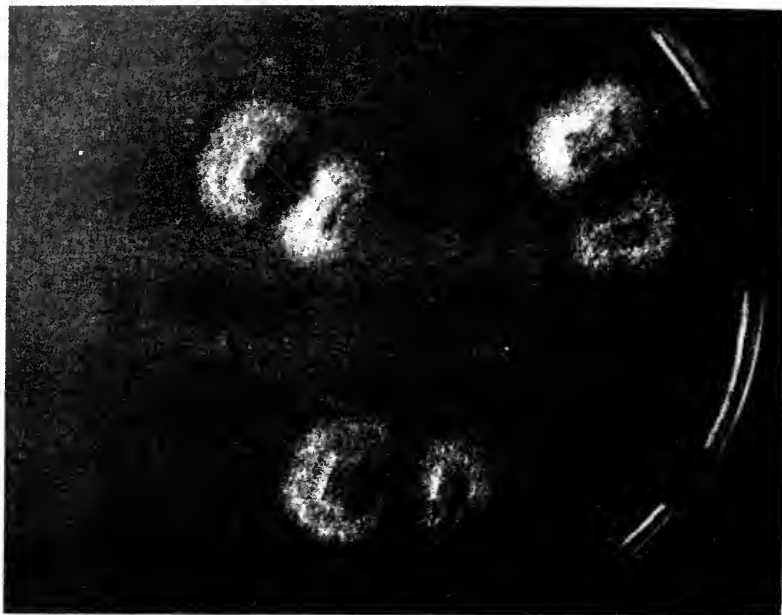


FIG. 134.—*Coprinus lagopus*. Method of pairing mycelia of various kinds with a view to observing hyphal fusions between them. The Petri dish contained cleared dung-agar. It was then inoculated with the following three pairs of mycelia: (*ab*) and (*Ab*), lower pair; (*ab*) and (*a'b'*), upper left-hand pair; (*ab*) and (*aB*), upper right-hand pair. The smaller mycelium of each pair is (*ab*). Photograph taken three days after inoculation. The two members of each pair, by means of hyphae in and in contact with the dung-agar, had met and had begun to form hyphal fusions with one another. Natural size.

mycelia, six belonging to each of the two sets. The two sets of mycelia may be distinguished as follows:

(*AB*), (*ab*), (*Ab*), (*aB*), (*AB*)+(*ab*), and (*Ab*)+(*aB*)
 (*A'B'*), (*a'b'*), (*A'b'*), (*a'B'*), (*A'B'*)+(*a'b'*), and (*A'b'*)+(*a'B'*)

In order to obtain evidence whether or not any two of the twelve mycelia, when paired together, can form hyphal fusions, twenty-one of the sixty-six mathematically possible pairings were made on dung-agar in Petri dishes (Fig. 134), and hyphal fusions were sought for in the zone where the two members of each pair

came into contact. The combinations which were investigated were the following :

A haploid mycelium with another haploid mycelium.

$(AB) \times (A'B')$, $(AB) \times (ab)$, $(AB) \times (Ab)$, $(AB) \times (aB)$.

$(ab) \times (a'b')$, $(ab) \times (Ab)$, $(ab) \times (aB)$.

$(Ab) \times (A'b')$, $(Ab) \times (aB)$.

$(aB) \times (a'B')$.

A haploid mycelium with a diploid mycelium.

$(AB) \times (AB)+(ab)$, $(AB) \times (Ab)+(aB)$,

$(ab) \times (AB)+(ab)$, $(ab) \times (Ab)+(aB)$,

$(Ab) \times (AB)+(ab)$, $(Ab) \times (Ab)+(aB)$,

$(aB) \times (AB)+(ab)$, $(aB) \times (Ab)+(aB)$.

A diploid mycelium with another diploid mycelium.

$(AB)+(ab) \times (A'B')+(a'b')$, $(AB)+(ab) \times (Ab)+(aB)$.

$(Ab)+(aB) \times (A'b')+(a'B')$.

In every one of the twenty-one pairings just indicated hyphal fusions were observed in the junction zone between the two mycelia. There is no reason to suppose that, if the other forty-five of the possible sixty-six combinations had been investigated, hyphal fusions would have been found lacking in them; and we may conclude from the observations actually made that, in general, in *Coprinus lagopus*, any mycelium can form hyphal fusions with any other mycelium.

A camera-lucida drawing showing the union of a diploid mycelium $(Ab)+(aB)$ with a haploid mycelium (Ab) is reproduced in Fig. 135.

The Diploidisation of a Haploid Mycelium by a Theoretically Incompatible Diploid Mycelium.—In the experiments hitherto described it has been shown that: (1) the haploid mycelia (AB) and (ab) can both be diploidised by the diploid mycelium $(AB)+(ab)$; and (2) the haploid mycelia (Ab) and (aB) can both be diploidised by the diploid mycelium $(Ab)+(aB)$.

In all the combinations :

$(AB) \times (AB)+(ab)$,

$(ab) \times (AB)+(ab)$,

$(Ab) \times (Ab)+(aB)$,

$(aB) \times (Ab)+(aB)$,

since the diploid mycelium contains nuclei of a sex opposite to that

of the nuclei in the haploid mycelium, it can be said that the

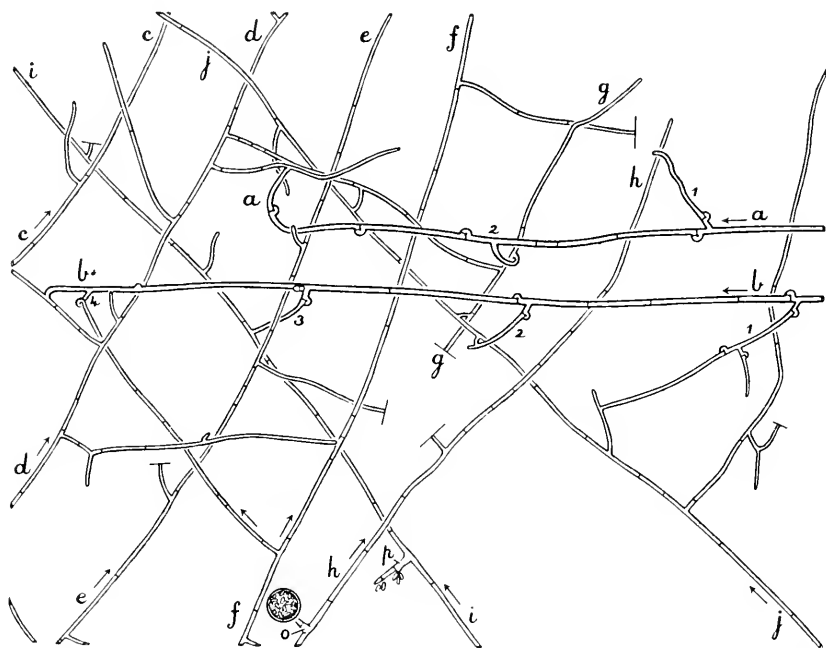


FIG. 135.—*Coprinus lagopus*. Camera-lucida drawing showing hyphal fusions between a diploid mycelium (Ab) + (aB) and a haploid mycelium (Ab). The two mycelia were set near to one another on a dung-agar plate; two days later they had grown into contact with one another, and then the drawing was made: $a-a$ and $b-b$, two radial hyphae of the diploid mycelium; $c-c$, $d-d$, $e-e$, $f-f$, $g-g$, and $h-h$, all radial hyphae of the haploid mycelium; $i-i$ and $j-j$, two long lateral tangential branches of radial hyphae of the haploid mycelium. The arrows indicate the direction of growth of the hyphae. A line across a hypha, as at the letter g in the centre of the illustration, indicates that a continuation of the hypha was seen but has not been drawn. The diploid hypha $a-a$: branch no. 1 is bent at the end as if about to fuse with $h-h$; branch no. 2 has fused with $g-g$; and the end has fused with a branch of $d-d$. The diploid hypha $b-b$: branch no. 1 has fused with a branch of $j-j$; branch no. 2 with another branch of $j-j$; branch no. 3 with $i-i$; branch no. 4 with a branch of $f-f$; and the end with a branch of $d-d$. Thus the diploid hyphae $a-a$ and $b-b$ have fused (anastomosed) in seven places with the haploid hyphae $c-c$ to $j-j$. The haploid hyphae have simple septa, no clamp-connexions, and wide-angled branching, while two of them, $h-h$ and $i-i$, each bear an oidiophore. The oidiophore o projects above the culture medium and bears oidia in a drop of liquid, while the oidiophore p is immersed in, and bears oidia in, a film of moisture on the surface of the culture medium. The haploid mycelium is becoming converted into a three-dimensional network: a branch of $d-d$ has fused with $g-g$, and a branch of that branch has fused with $j-j$; $j-j$ has also fused with $g-g$. The diploid hyphae bear clamp-connexions but no oidiophores, and they have a narrow-angled mode of branching. Drawn by A. H. R. Buller and Ruth Macrae. Magnification, 88.

haploid mycelium becomes diploidised by a diploid mycelium with which it is *theoretically compatible*.

The question may now be asked: can a haploid mycelium be diploidised by a diploid mycelium with which it is *theoretically incompatible*? Can (1) the haploid mycelia (AB) and (ab) be diploidised by the diploid mycelium $(Ab)+(aB)$; and can (2) the haploid mycelia (Ab) and (aB) be diploidised by the diploid mycelium $(AB)+(ab)$? In the combinations:

$$(AB) \times (Ab)+(aB),$$

$$(ab) \times (Ab)+(aB),$$

$$(Ab) \times (AB)+(ab),$$

$$(aB) \times (AB)+(ab),$$

where the diploid mycelium does not contain nuclei of a sex opposite to that of the nuclei in the haploid mycelium, can the diploid mycelium effect any sexual change in the haploid mycelium? An attempt to answer these questions was made by means of a number of experiments which will now be described.

First Series of Experiments. As indicated in Table V, four large haploid mycelia (AB) , (ab) , (Ab) , and (aB) were each inoculated with a theoretically incompatible diploid mycelium. Thus, for example, the haploid mycelium (AB) was inoculated with a diploid mycelium $(Ab)+(aB)$ which did not contain any (ab) nuclei and which, therefore, from the *prima-facie* theoretical point of view, should not be able to effect its diploidisation.

As a control for the four illegitimate pairings, four legitimate pairings of the type $(AB) \times (AB)+(ab)$ were also made, and the results of these pairings are indicated in the Table.

For convenience in reference, combinations of the type $(AB) \times (AB)+(ab)$ or $(Ab) \times (Ab)+(aB)$ will be called *legitimate combinations* and combinations of the type $(AB) \times (Ab)+(aB)$ or $(Ab) \times (AB)+(ab)$ *illegitimate combinations*.

An inspection of Table V shows that in the *illegitimate* combinations: (1) the diploid inoculum $(Ab)+(aB)$ brought about no change in the haploid mycelium (ab) but caused the haploid mycelium (AB) to become diploid (as shown by mode of branching and development of clamp-connexions) one-sixth of the way around its periphery; and (2) the diploid inoculum $(AB)+(ab)$ brought about no change in the haploid mycelium (aB) but caused the haploid mycelium (Ab) to become diploid one-half of the way around its periphery. In the *legitimate* combinations, as shown by

a further inspection of Table V, all the haploids were completely diploidised by their diploid inocula.

TABLE V.

Results of inoculating Large Haploid Mycelia (1) with Diploid Mycelia theoretically incompatible with them and (2) with Diploid Mycelia theoretically compatible with them.

No.	Combinations.		Results of pairing : effect of the diploid on the haploid mycelium.
	large haploid	small diploid inoculum	
1	illegitimate	$(AB) \times (Ab) + (aB)$	Diploidisation of haploid (AB) one-sixth way around.
2		$(ab) \times (Ab) + (aB)$	No change in haploid (ab) .
3		$(Ab) \times (AB) + (ab)$	Diploidisation of haploid (Ab) one-half way around.
4		$(aB) \times (AB) + (ab)$	No change in haploid (aB) .
5	legitimate	$(AB) \times (AB) + (ab)$	Complete diploidisation of the haploid all around its periphery.
6		$(ab) \times (AB) + (ab)$	
7		$(Ab) \times (Ab) + (aB)$	
8		$(aB) \times (Ab) + (aB)$	

It is to be noted that, in the illegitimate combinations $(AB) \times (Ab) + (aB)$ and $(Ab) \times (AB) + (ab)$, the diploid mycelia which were produced by the conversion of the haploid mycelia (AB) and (Ab) developed at the periphery of these haploid mycelia in a very "patchy" manner (*cf.* Fig. 136), and not evenly as happens in a legitimate combination such as $(AB) \times (AB) + (ab)$ or $(Ab) \times (Ab) + (aB)$. The same phenomenon—"patchiness" in the production of the diploid mycelium on the haploid—has been noticed whenever such a diploid mycelium has been produced as a result of an illegitimate union. It may be that "patchiness" is an indication that the nuclei which move out of the diploid inoculum into the large haploid mycelium are not entirely compatible with all the nuclei of the haploid mycelium.

The four experiments with illegitimate combinations recorded in

Table V were made a second and then a third time, with essentially the same results as before. In each of the new sets of experiments,

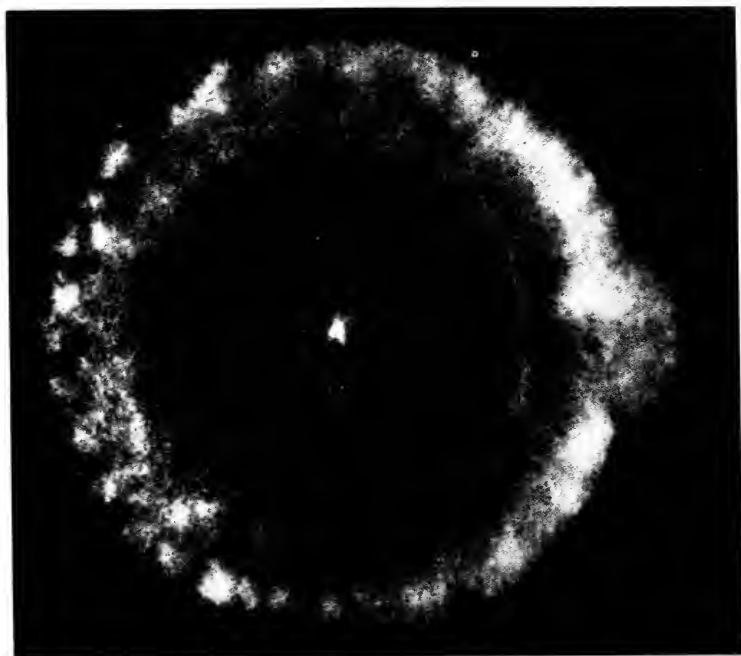


FIG. 136.—*Coprinus lagopus*. An illegitimate mating of a large haploid mycelium with a small diploid inoculum resulting in the diploidisation of the haploid mycelium; to show the "patchiness" of the diploid mycelium into which the haploid mycelium has been converted. A haploid mycelium (AB) was allowed to grow on cleared dung-agar in a Petri dish until it was 6.2 cm. in diameter. It was then inoculated at its periphery (right-hand side) with a tiny hyphal mass of a diploid mycelium (Ab)+(aB). The diploid inoculum soon began to diploidise the large haploid mycelium; and, four days after inoculation, hyphae bearing clamp-connexions could be observed all around the periphery of the large originally-haploid central mycelium. The photograph was then taken. The diploid mycelium around the central mycelium is very "patchy" and therefore contrasts with the diploid mycelium produced in experiments made in a similar manner but with legitimate combinations of haploids and diploids (cf. Figs. 126 and 127). Natural size.

the combinations (AB) \times (Ab)+(aB) and (Ab) \times (AB)+(ab) again yielded a partial diploidisation of the haploid mycelia, whereas in the combinations (ab) \times (Ab)+(aB) and (aB) \times (AB)+(ab) the haploid mycelia remained in the haploid condition.

We may ask: how in Experiments No. 1 and No. 3 of Table V

did the diploid inoculum manage partially to diploidise the large haploid mycelium? Let us consider the diploidisation of the haploid mycelium (Ab) by the diploid inoculum (AB)+(ab). It seemed possible that the union which had taken place was essentially of the same nature as the "Durchbrechungskopulationen" recorded as sometimes occurring in haploid combinations such as (Ab) \times (AB) or (aB) \times (ab) by Brunswik¹ in *Coprinus picaceus* and, more recently, by Oort² in *Coprinus fimetarius* (the *C. lagopus* of this chapter). In certain of these combinations Oort found that patches in the united mycelia could be observed in which clamp-connexions were present, but that this apparently normal diploid mycelium, when transferred to a new culture medium, produced *haploid* and not diploid fruit-bodies. He states that some of the basidia produced from a combination (AB) \times (Ab) bore four spores (AB), (AB), (AB), and (AB) and other basidia four spores (Ab), (Ab), (Ab), and (Ab), so that there were only *two* kinds of spores. On this account he is inclined to regard the fruit-body which produced these two kinds of spores as built up of two kinds of hyphae, (AB) and (Ab), and the pilei as "haploid chimaerae."

In view of Oort's investigation of "Durchbrechungskopulationen," which has just been recorded, it seemed possible, in the combination (Ab) \times (AB)+(ab) of Table V, that either a nucleus (AB) or a nucleus (ab) had, at the beginning of the diploidisation process, moved out of the diploid inoculum into the large haploid mycelium (Ab) and that its progeny had associated themselves with (Ab) nuclei, so as to form a mycelium producing clamp-connexions and having the constitution (Ab)+(AB) or (Ab)+(ab). An analysis of the sex-factors of the mycelium in question was therefore undertaken.

A piece of the clamp-connexion-bearing mycelium produced on the large (Ab) mycelium (Experiment No. 3 of Table V) at a place 5.5 cm. distant from the inoculum was removed from the dung-agar Petri dish and placed on sterilised horse dung in a wide test-tube

¹ H. Brunswik, "Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*," *Botanische Abhandlungen* herausgeg. v. Goebel, Heft V, 1924, pp. 85-90.

² A. J. P. Oort, "The Sexuality of *Coprinus fimetarius*," *Proceedings of the Koninklijke Academie van Wetenschappen te Amsterdam*, vol. xxxii, 1929, pp. 1-6.

(3 × 1 inches). The mycelium on its new culture medium grew well and, after about ten days, yielded a rather large fruit-body which shed an abundance of spores. Some of the spores were sown separately on dung-agar and thus ten monosporous mycelia were obtained. These mycelia were then paired with four tester mycelia (*AB*), (*ab*), (*Ab*), and (*aB*) of the original parental strain, with results which are shown in Table VI.

TABLE VI.

Coprinus lagopus. Pairings of Four Original Haploid Mycelia with Ten Monosporous Mycelia derived from Ten Spores of a Fruit-body produced on a Large Haploid Mycelium (*Ab*) after this Mycelium had been diploidised by a Diploid Inoculum (*AB*)+(*ab*).

New haplonts from $(Ab) \times (AB) + (ab)$													
AB			ab			Ab		aB					
1	3	8	2	7	10	4	5	9	6				
Original haplonts {	{	AB	2	—	—	—	+	+	+	—	—	—	+
		ab	6	+	+	+	—	—	—	—	—	—	+
		Ab	1	—	—	—	—	—	—	—	+	+	
		aB	9	—	—	—	—	—	—	+	+	—	+

From Table VI, it will be seen that the ten monosporous mycelia derived from the fruit-body in the test-tube fall, with one exception, into four groups identical with those of the four testers, namely, (*AB*), (*ab*), (*Ab*), and (*aB*). The exceptional mycelium No. 6, shown in the last column of Table VI, produced clamp-connexions when paired with *all* the four testers; and this result was again obtained when No. 6 was mated a second time with all the four testers. Whether or not mycelium No. 6 was a mutation could only be decided by further experiments, but these experiments were not undertaken.

The analysis just recorded does not support the view that the diploidisation of the mycelium (*Ab*) in Experiment No. 3 of Table V was due to a "Durchbrechungskopulation" of the kind described

by Oort ; for, if it had been, the spores produced from the test-tube fruit-body should have fallen into two groups only, *i.e.* (*Ab*) and (*AB*) or (*Ab*) and (*ab*), and not into four groups (*AB*), (*ab*), (*Ab*), and (*aB*), plus an extra exceptional group represented by the spore which produced mycelium No. 6.

The analysis given in Table VI may possibly be explained as follows. Somehow or other, as a result of placing the diploid inoculum (*AB*)+(*ab*) in contact with the haploid mycelium (*Ab*), a nucleus (*aB*) came into existence and thus, after division, provided mates for the (*Ab*) nuclei in the (*Ab*) mycelium. Did a nucleus (*AB*) or a nucleus (*ab*) of the inoculum alter one of its factors so as to become a nucleus (*aB*) ? Or, to meet the emergency when the haploid and diploid mycelia fused, did the (*AB*) and (*ab*) nuclei of some conjugate pair in the diploid mycelium fuse together and the fusion nucleus then undergo a reduction division or reduction divisions to produce the four types of nuclei (*AB*), (*ab*), (*Ab*) and (*aB*) so that a nucleus (*aB*) could pass into the large haploid mycelium (*Ab*) ?

As bearing on the answer which may be given to the questions just raised, the following remarks may be made. Nuclear fusion and reduction in a diploid *mycelium* seems, *a priori*, a rather unlikely phenomenon as, hitherto, in the Hymenomycetes, these processes have been found to occur only in the basidia. Furthermore, if, in the particular case under discussion, a nucleus (*aB*) had been produced by nuclear fusion and nuclear reduction in one of the hyphae of the diploid mycelium, there seems no reason why such a nucleus should not, on entering the large (*Ab*) mycelium, have behaved like other (*aB*) nuclei, *e.g.* those in Experiment No. 7 of Table V, and have brought about a complete, instead of only a partial, diploidisation of the (*Ab*) mycelium. The fact that the (*Ab*) mycelium became diploidised only one-half the way around its periphery suggests that the (*aB*) nuclei were not fully compatible with the (*Ab*) nuclei. Finally, the solution of the problem in hand must await further analysis of the clamp-connexion-bearing mycelia produced in a new series of illegitimate combinations of the type (*Ab*) \times (*AB*)+(*ab*). Up to the present, the writer has not been able to undertake the work suggested.

After it had been observed in three successive sets of experiments that the diploid mycelium $(AB)+(ab)$ had diploidised (caused clamp-connexions to develop on) the haploid mycelium (Ab) and that the diploid mycelium $(Ab)+(aB)$ had diploidised the haploid mycelium (AB) , it was deemed advisable to re-mate the original haploids (AB) , (ab) , (Ab) , and (aB) from which the two diploid mycelia had been made, to find out whether or not they would still mate with one another in the usual manner. Therefore the following six combinations of equally large mycelia were made on dung-agar in Petri dishes :

<i>Legitimate.</i>	<i>Illegitimate.</i>
$(AB) \times (ab)$	$(AB) \times (Ab)$
$(Ab) \times (aB)$	$(AB) \times (aB)$
	$(ab) \times (Ab)$
	$(ab) \times (aB)$

It was found that, in the legitimate combinations, both of the haploid mycelia soon became completely diploid whereas, in the illegitimate combinations, both of the haploid mycelia remained completely haploid.

Since the diploid mycelium $(AB)+(ab)$ was able to diploidise the haploid mycelium (Ab) , one might have expected that one or other of the components which were employed to form the diploid, *i.e.* the haploid mycelium (AB) or the haploid mycelium (ab) , would have been able to diploidise the haploid mycelium (Ab) ; but this supposition, as we have seen, did not receive any experimental support.

Since it was found that a haploid mycelium (Ab) could be diploidised by the diploid mycelium $(AB)+(ab)$, but not by the haploid mycelium (AB) or by the haploid mycelium (ab) , the following question arose: in general, in illegitimate combinations, *does a diploid mycelium act on a haploid mycelium in a manner which is different from that of either of the two haploid mycelia which produced it?* An attempt to answer this question will be made in the third and last series of experiments described in this Section.

Second Series of Experiments. Since the first series of experiments had yielded such an unexpected result, namely, the diploidi-

sation of certain large haploid mycelia by certain diploid mycelia in illegitimate combinations, a second series of experiments similar to the first series, but starting with a new set of haploid mycelia, was undertaken.

A large haploid mycelium (AB) inoculated with a diploid mycelium (AB) + (ab) had become completely diploidised, and the diploid mycelium, after transference to sterilised horse dung, developed a fruit-body.

Some of the spores of the fruit-body were germinated individually. The monosporous mycelia thus obtained were then paired in all possible combinations, with the result that they were found to fall into the usual four groups (AB), (ab), (Ab), and (aB). Four of these mycelia, one from each group, were then used to make up the two possible kinds of diploid mycelia (AB) + (ab) and (Ab) + (aB). Finally, a haploid mycelium was paired with a diploid mycelium in each of the four possible illegitimate combinations as follows :

$$\begin{aligned} & (AB) \times (Ab) + (aB), \\ & (ab) \times (Ab) + (aB), \\ & (Ab) \times (AB) + (ab), \\ & (aB) \times (AB) + (ab). \end{aligned}$$

Each of the haploid mycelia was allowed to grow until it was several centimetres in diameter, and then it was inoculated at its periphery with a tiny fragment of the diploid mycelium.

The results of these experiments were even more surprising than those obtained in the first series of experiments ; for, in *every* combination the large haploid mycelium became diploidised completely or almost completely all around its periphery (shown by diploid mode of branching and presence of clamp-connexions).

The discovery made in the first two series of experiments that, in illegitimate combinations, a diploid mycelium may sometimes or often diploidise a haploid mycelium, led the writer to make a third and last series of experiments which will now be recorded.

Third Series of Experiments. This series of experiments was made with a view to deciding the question raised at the end of the discussion of the results of the first series of experiments : in general, in illegitimate combinations, does a diploid mycelium act

on a haploid mycelium in a manner different from that of either of the two haploid mycelia which produced it ?

Four haploid mycelia (AB), (ab), (Ab), and (aB) and two diploid mycelia (AB)+(ab) and (Ab)+(aB) required for making the experiments were obtained as follows. A large haploid mycelium (AB) was inoculated with a tiny fragment of a diploid mycelium (AB)+(ab). The (AB) mycelium became diploidised (as indicated by the appearance of clamp-connexions and a change from the haploid to the diploid mode of branching in all the peripheral hyphae) in about three days. A piece of the diploidised mycelium was placed on sterilised horse dung where it grew well and soon produced fruit-bodies. From one of the fruit-bodies a spore-deposit was obtained. Ten of the spores of this spore-deposit, after being sown on dung-agar, yielded as many haploid mycelia. These ten mycelia were then paired in all possible ways (*cf.* Table III, p. 236) with the result that it was found that in the ten mycelia there were representatives of all the four possible kinds (AB), (ab), (Ab), and (aB). Four of these mycelia, one of each of the kinds (AB), (ab), (Ab), and (aB), were chosen as the four haploid mycelia required for making the experiments about to be described. Pieces of these four mycelia were then paired on two dung-agar plates in the combinations (AB) \times (ab) and (Ab) \times (aB); and thus the two required diploid mycelia (AB)+(ab) and (Ab)+(aB) were obtained.

The four haploid mycelia (AB), (ab), (Ab), and (aB) and the two diploid mycelia (AB)+(ab) and (Ab)+(aB) obtained in the manner just described were grown separately on as many dung-agar plates. As soon as they had attained a sufficiently large size they were used to inoculate twenty-four freshly-poured dung-agar plates: six plates were inoculated with the (AB) mycelium, six with the (ab) mycelium, six with the (Ab) mycelium, and six with the (aB) mycelium. The twenty-four plates were then placed under glass covers and left there for a few days until each of the twenty-four mycelia had attained a diameter of about 4.5 cm. Each of these twenty-four large mycelia was then inoculated at its periphery with a small fragment of one of the mycelia (AB), (ab), (Ab), (aB), (AB)+(ab), and (Ab)+(aB) which were growing in the plates referred to at the beginning of this paragraph.

The twenty-four large haploid mycelia were inoculated as follows: (1) one of each kind with a legitimate haploid mycelium; (2) one of each kind with a legitimate diploid mycelium; (3) one

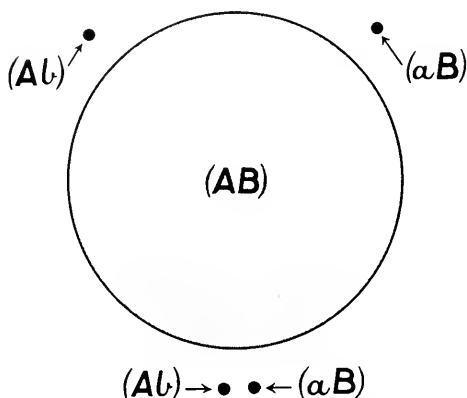


FIG. 137.—*Coprinus laqopus*. Method of making illegitimate combinations in one set of experiments. The large haploid mycelium (AB), growing on dung-agar in a Petri dish, has attained a diameter of 4.5 cm. At a little distance away from its periphery have just been set four inocula, the size and position of which are indicated by the black spots. The two lower inocula (Ab) and (aB) quickly met and diploidised one another, and this diploid mycelium, on coming into contact with the large central (AB) mycelium, soon began to diploidise the (AB) mycelium. The upper isolated (Ab) and (aB) inocula, which were too far distant from one another to meet and fuse, on coming into contact with the large central (AB) mycelium failed to diploidise the (AB) mycelium; but, eventually, they became diploidised by the large central (AB) mycelium after this had itself become diploidised. Natural size.

of each kind with an illegitimate haploid mycelium; (4) one of each kind with another illegitimate haploid mycelium; (5) one of each kind with an illegitimate diploid mycelium; and, finally, (6) one of each kind with two separated illegitimate haploid mycelia and with an illegitimate diploid mycelium made by combining two illegitimate haploid mycelia at the periphery of the large haploid mycelium. Thus six kinds of combinations were made with each of the four kinds of large haploid mycelia (AB), (ab), (Ab) and (aB).

The sixth kind of combination requires to be described in greater detail, as results obtained from it are of considerable importance for the solution of the problem under investigation.

It may best be described by reference to one of the actual combinations: the large haploid mycelium (AB) inoculated with the two illegitimate haploid mycelia (Ab) and (aB) and with the illegitimate diploid mycelium (Ab) + (aB). When the large haploid mycelium (AB) had attained a diameter of about 4.5 cm., it was inoculated at its periphery, as shown in Fig. 137, at three widely separated places: at one place was set down a fragment of an (Ab) mycelium,

at another place was set down a fragment of an (aB) mycelium, while at the third place was set down a fragment of an (Ab) mycelium and a fragment of an (aB) mycelium side by side and close together. The two fragments of mycelia (Ab) and (aB) lying close together, after beginning to grow, quickly came into contact with one another and thus built up the illegitimate diploid mycelium (Ab)+(aB) on the plate. The two fragments of mycelium (Ab) used for inoculating the mycelium (AB) were taken out of one and the same plate at one and the same time; and a similar procedure was followed for the two fragments of the mycelium (aB). The chances of error in comparing the effect on the large haploid mycelium (AB) of the illegitimate diploid mycelium (Ab)+(aB) with the effect of the two illegitimate haploid mycelia (Ab) and (aB) was thus reduced to a minimum.

The nature of all the twenty-four combinations and the results which they gave are set forth in Tables VII, VIII, and IX.

TABLE VII.

Legitimate Combinations: the Effect produced on a Large Haploid Mycelium by a Small Haploid or Diploid Inoculum.

No.	Combinations		Results
	Large haploid	Small inoculum	
1	(AB)	(ab)	In every combination the small inoculum diploidised the large haploid mycelium rapidly and completely.
2	(AB)	(AB)+(ab)	
3	(ab)	(AB)	
4	(ab)	(AB)+(ab)	
5	(Ab)	(aB)	
6	(Ab)	(Ab)+(aB)	
7	(aB)	(Ab)	
8	(aB)	(Ab)+(aB)	

The results embodied in Table VII indicate that, in the eight legitimate combinations, the small haploid or diploid inoculum

diploidised the large mycelium rapidly and completely. This, in view of the results of experiments described earlier in this chapter, was to be expected.

TABLE VIII.

Illegitimate Combinations : Comparison of the Effect produced on a Large Haploid Mycelium by (1) two different Haploid Inocula and by (2) a Diploid Inoculum.

No.	Kind of inoculum	Combinations		Results of pairing: effect of the inoculum on the large haploid mycelium
		Large haploid	Small inoculum	
1	haploid	(AB)	(Ab)	(AB) remained haploid, except for a few hyphae at the junction with (Ab).
2	haploid	(AB)	(aB)	(AB) remained haploid.
3	diploid	(AB)	(Ab) + (aB)	(AB) converted slowly and patchily into a diploid mycelium.
4	haploid	(ab)	(Ab)	(ab) remained haploid.
5	haploid	(ab)	(aB)	(ab) remained haploid.
6	diploid	(ab)	(Ab) + (aB)	(ab) converted slowly and patchily into a diploid mycelium.
7	haploid	(Ab)	(AB)	(Ab) remained haploid.
8	haploid	(Ab)	(ab)	(Ab) remained haploid, except for a few hyphae at the junction with (ab).
9	diploid	(Ab)	(AB) + (ab)	(Ab) converted slowly and patchily into a diploid mycelium.
10	haploid	(aB)	(AB)	(aB) remained haploid.
11	haploid	(aB)	(ab)	(aB) remained haploid.
12	diploid	(aB)	(AB) + (ab)	(aB) converted slowly and patchily into a diploid mycelium.

In Table VIII are shown the results obtained in eight illegitimate combinations where the inoculum was haploid and in four where the inoculum was diploid. In six of the combinations, Nos. 2, 4, 5, 7, 10, and 11, the haploid inoculum effected no change in the large haploid mycelium. In two of the combinations, Nos. 1 and 8,

the haploid inoculum caused clamp-connexions to appear on a few hyphae situated at the junction between itself and the large haploid mycelium; but the diploidisation did not spread laterally farther, so that the large haploid mycelium remained almost completely haploid. It is therefore clear that the haploid inocula diploidised the large haploid mycelium either not at all or to an extremely slight degree. The diploid inocula exerted a far more powerful effect on the large haploid mycelium than the haploid inocula; for, in each of the four combinations in which a diploid inoculum was employed, Nos. 3, 6, 9, and 12, the diploid inoculum converted the haploid mycelium into a diploid mycelium, although slowly and patchily as compared with what happens in a legitimate combination of a large haploid mycelium and a diploid inoculum (*cf.* Nos. 2, 4, 6, and 8 in Table VII).

The twelve experiments of Table VIII are set out in four series of three each. In each series of three are shown the results obtained by subjecting one kind of large haploid mycelium first to one haploid inoculum, then to another haploid inoculum of opposite sex, and then to a diploid inoculum which originated by the interaction of two mycelia like the two haploid inocula. Thus, for example, in the first three experiments set out in the Table, we can compare the effect on a large haploid mycelium (AB) of a diploid inoculum (Ab)+(math>aB) with the effect of its two components, the haploid inocula (Ab) and (aB) employed separately.

The conclusion to which a study of the results shown in Table VIII points is that, in illegitimate combinations, as a rule, a large haploid mycelium can be converted into a diploid mycelium by a diploid inoculum, but not by either of two haploid inocula taken from the two haploid mycelia which were mated to provide the diploid inoculum.

That a diploid inoculum is far more active in diploidising a large haploid mycelium than either of its two haploid components is again shown by a study of the four experiments recorded in Table IX. The diploidisation process in each of the four experiments (*cf.* Fig. 137) took place in three steps: (1) first, the two bits of haploid mycelia set side by side quickly diploidised one another and thus formed a diploid inoculum for the large haploid

mycelium ; (2) then this diploid inoculum diploidised the large haploid mycelium progressively from the side in contact with the inoculum all around the periphery to the opposite side (Fig. 138) ; and, finally, (3) the large haploid mycelium, after becoming diploidised, diploidised both of the isolated haploid inocula. In each of the four experiments, the large haploid mycelium was diploidised by the diploid inoculum but not by the two isolated haploid inocula.

TABLE IX.

Illegitimate Combinations : Comparison of the Effect produced on a Large Haploid Mycelium by Three Simultaneously-applied Widely-separated Inocula, Two Haploid and one Diploid.

No.	Combinations				Results, as indicated by the development of clamp-connexions and a change from the haploid to the diploid mode of branching
	Large haploid	The three small inocula			
		Haploid	Haploid	Diploid, formed <i>in situ</i> by two haploids set close together	
1	(AB)	(Ab)	(aB)	(Ab)+(aB)	In each of the four experiments : (1) the two haploid mycelia set close together quickly diploidised each other to form the diploid inoculum ; (2) then the diploid inoculum diploidised the large haploid mycelium ; and, finally, (3) the large haploid, after becoming diploidised, diploidised the two separate haploid inocula.
2	(ab)	(Ab)	(aB)	(Ab)+(aB)	
3	(Ab)	(AB)	(ab)	(AB)+(ab)	
4	(aB)	(AB)	(ab)	(AB)+(ab)	

Some time-data recorded for Experiment No. 3 of Table IX are as follows. The experiment was started about 1 P.M. on May 8. Two days after inoculation, the (AB) and (ab) mycelial fragments which had been set close together were found to have diploidised one another, so that a diploid inoculum for the large (Ab) mycelium had become established. Three days after inoculation diploid hyphae were observed to be present at intervals all around the large haploid mycelium (Ab), but none could be detected in the two isolated inocula (AB) and (ab). Four days after inoculation, diploid hyphae were observed all around the periphery of each of

the isolated inocula (AB) and (ab). The time-data for Experiments Nos. 1, 2, and 4 of Table IX resembled those just given for Experiment No. 3.

Concluding remarks on the three series of experiments. Assuming

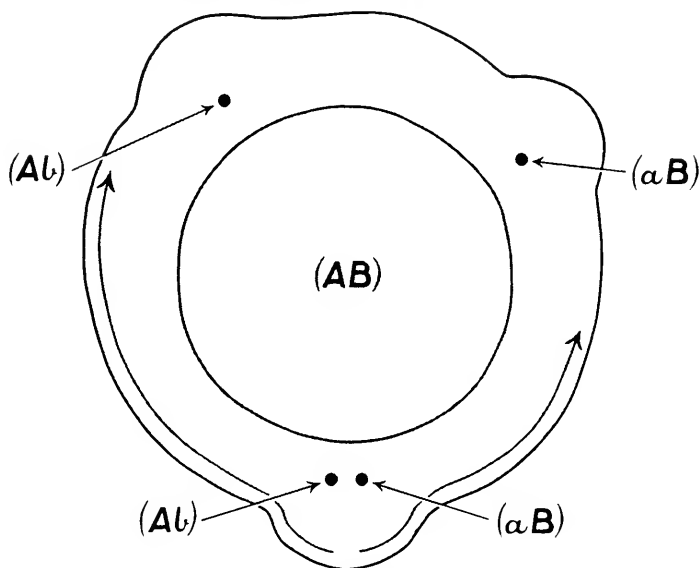


FIG. 138.—*Coprinus lagopus*. Progress of the diploidisation process in an illegitimate combination of a large haploid mycelium (AB) inoculated at three places on its periphery with: an isolated haploid mycelium (Ab); an isolated haploid mycelium (aB); and a pair of haploid mycelia (Ab) and (aB), set close together so that they could soon diploidise one another and so build up *in situ* a diploid inoculum for the (AB) mycelium (cf. Fig. 137). The drawing, made from wax-pencil tracings on the base of the Petri dish, shows the progress of the experiment on the third day after inoculation. The lower pair of inocula (Ab) and (aB) first diploidised one another, and now the diploid mycelium so produced is diploidising the large central (AB) mycelium in the directions shown by the curved arrows. The peripheral hyphae of the (AB) mycelium show clamp-connexions as far from the lower pair of inocula as is represented by the heads of the curved arrows. Later on, when the (AB) mycelium became further diploidised, it diploidised the two upper haploid mycelia (Ab) and (aB). Drawn by A. H. R. Buller and Ruth Macrae. Natural size.

that the development of clamp-connexions and a change to narrow-angled branching by a haploid mycelium are indications that the haploid mycelium is becoming or has become diploidised, the results of the three sets of experiments recorded in this Section show quite clearly that, in illegitimate combinations, a diploid mycelium

differs from the two haploid mycelia from which it has been derived in that, as a rule, when used as an inoculum, it is able to diploidise a large haploid mycelium, whereas, as a rule, when they are used as inocula, they are not able to do so.

Unfortunately, owing to the fact that the writer was leaving his laboratory for eight months, it was impossible for him to analyse the diploidised haploid mycelia produced in the experiments recorded in Tables VIII and IX for their sex-factors. Some of these mycelia were transferred to sterilised horse dung and spore-deposits were obtained from the fruit-bodies which they produced ; but the work had to be stopped at this point. It was therefore not possible to germinate the spores, to obtain monosporous mycelia, and to pair these mycelia with one another and with the primary mycelia (AB) , (ab) , (Ab) , and (aB) . Work of this kind is required to inform us whether the diploidised haploid mycelia were normal diploid mycelia of the types $(AB)+(ab)$ and $(Ab)+(aB)$ or whether they were abnormal mycelia of the types $(AB)+(aB)$, $(AB)+(Ab)$, $(ab)+(aB)$, and $(ab)+(Ab)$. It is true that the analysis (p. 251) of the diploid mycelium produced on a mycelium (Ab) by a diploid inoculum $(AB)+(ab)$ in Experiment No. 3 of Table V indicated that the diploidised mycelium had the constitution $(Ab)+(aB)$ and not $(Ab)+(AB)$ or $(Ab)+(ab)$; but this analysis, unfortunately, stands alone, and it is possible that other similar analyses, yet to be made, may not yield the same kind of result. In illegitimate combinations of a haploid and a diploid mycelium, it is therefore still an open question whether the diploidisation of the haploid mycelium is due simply to "Durchbrechungskopulationen" of the kind already discussed earlier in this Section or is due to the production by the diploid mycelium of one or more nuclei of a sex opposite to that of the nuclei of the haploid mycelium. As a concrete example of this question, we desire to know whether, in the combination $(AB) \times (Ab)+(aB)$, when the mycelium (AB) becomes diploidised, does the diploid mycelium send one or more (Ab) or one or more (aB) nuclei into the (AB) mycelium so that the pairs of nuclei in each cell of the diploidised (AB) mycelium are $(AB)+(Ab)$ or $(AB)+(aB)$, or do the (Ab) and (aB) nuclei of the diploid mycelium when stimulated by the (AB) mycelium interact

in some way or change individually in some way so that they can give rise to one or more (ab) nuclei which can pass into the (AB) mycelium and diploidise it in the normal manner ?

If it should be found that, in an illegitimate combination of a haploid mycelium with a diploid mycelium, the diploidisation of the haploid mycelium is due simply to "Durchbrechungskopulationen," another question suggests itself : how is it that, in illegitimate combinations, a large haploid mycelium can be diploidised by a diploid inoculum but not by either of the components of the diploid inoculum ? To cite a concrete example of this question : how is it that a large mycelium (AB) can be diploidised by a diploid inoculum (Ab)+(aB), but not by a haploid inoculum (Ab) or by a haploid inoculum (aB) ?

In the four experiments recorded in Table IX, as we have seen, the diploid inoculum diploidised the large haploid mycelium and then the diploidised large haploid mycelium diploidised both of the isolated haploid inocula. Progressive diploidisation of this kind doubtless takes place under natural conditions in horse-dung balls ; for, in these substrata, haploid and diploid mycelia of *Coprinus lagopus* must often meet one another and form all sorts of illegitimate combinations. The study of illegitimate combinations between haploid and diploid mycelia is therefore not merely of genetic interest, but is of importance for elucidating the life-history of *Coprinus lagopus* and other Hymenomycetes.

The Biological Significance of the Diploidisation of a Haploid Mycelium by a Diploid Mycelium.—In previous pages of this chapter it has been demonstrated that, in *Coprinus lagopus*, a typical Hymenomycete, a diploid mycelium (AB)+(ab) can readily diploidise the haploid mycelia (AB) and (ab), and that a diploid mycelium (Ab)+(aB) can readily diploidise the haploid mycelia (Ab) and (aB) ; and evidence was adduced to show that, in these so-called *legitimate* combinations, the haploid mycelium becomes diploidised by nuclei of opposite sex derived from the diploid mycelium. Thus, in the combination (AB) \times (AB)+(ab), diploidisation of the haploid mycelium (AB) is effected by one or more (ab) nuclei, derived from the conjugate nuclei of the diploid mycelium (AB)+(ab). Furthermore, it was shown that in the so-called

illegitimate combinations, e.g. $(AB) \times (Ab) + (aB)$ and $(Ab) \times (AB) + (ab)$, diploidisation of the haploid mycelium by the diploid mycelium (as judged by the development of clamp-connexions on the haploid mycelium and in one instance by the sex-factor analysis of the clamp-connexion-bearing mycelium) often, if not always, takes place; but, it was pointed out that the exact explanation of the diploidisation of a haploid mycelium in an illegitimate combination of a haploid mycelium and a diploid mycelium must await further investigation.

While, as yet, in *Coprinus lagopus*, in all so-called illegitimate combinations of haploid and diploid mycelia, when the diploid mycelium diploidises the haploid mycelium it still remains to be determined whether or not the diploidisation process is normal in the sense that each diploidised cell comes to contain two nuclei of opposite sex $(AB) + (ab)$ or $(Ab) + (aB)$, we are justified in affirming that in all so-called legitimate combinations of haploid and diploid mycelia, namely, the following :

$$\begin{aligned} &(AB) \times (AB) + (ab), \\ &(ab) \times (AB) + (ab), \\ &(Ab) \times (Ab) + (aB), \\ &(aB) \times (Ab) + (aB), \end{aligned}$$

the diploidisation of the haploid mycelium is effected as normally, smoothly, and rapidly as it is in the so-called legitimate combinations of two haploid mycelia :

$$\begin{aligned} &(AB) \times (ab), \\ &(Ab) \times (aB). \end{aligned}$$

This being so, we must admit that, under natural conditions, in *Coprinus lagopus* (and probably in the Hymenomycetes generally) there are *two kinds of normal matings*: (1) between two haploid mycelia of opposite sex; and (2) between a diploid mycelium and an appropriate haploid mycelium. The first of these two kinds of matings resembles in principle the mating of two haploid gametes in animals and most plants, while the second finds no parallel in animals and most plants. In no animal or plant does a fertilised egg fertilise an unfertilised egg.

The fact that, in legitimate combinations of a haploid mycelium

and a diploid mycelium, the diploid mycelium diploidises the haploid, has an important bearing on the course of the life-history of *Coprinus lagopus* as the fungus grows under natural conditions.

Coprinus lagopus is coprophilous, and its relations with its environment may be thus briefly summarised: the fruit-bodies appear on horse dung in fields and pastures; the spores liberated from the pilei in great numbers are carried off by the wind and are deposited on herbage; grazing horses eat the herbage with the spores attached thereto; the spores pass down the alimentary canal of the horses concerned unharmed, and so are deposited in the solid faeces where they germinate and produce a mycelium which, in the course of 8-15 days, may give rise to new fruit-bodies.

The number of spores of *Coprinus lagopus* which come to be embedded in a single horse-dung ball deposited in a pasture must be very variable; but, doubtless, it is often very great, amounting to more than one hundred or even one thousand. When many spores of our fungus are embedded in a single ball, the haploid mycelia produced by them must of necessity come into contact with one another as they develop. Since it has been shown (p. 245) that any two mycelia of *Coprinus lagopus* can form hyphal fusions with one another, we are justified in supposing that, when a dung-ball contains many spores, the monosporous mycelia which the spores produce must, on coming into contact with one another, unite to form a single compound mycelium.

The mycelium of *Coprinus lagopus* in a dung-ball produces fruit-body rudiments and fruit-bodies only at the exterior surface of the dung-ball, and never in the dung-ball's interior.

Coprinus lagopus is able to produce haploid fruit-bodies on a haploid mycelium and diploid fruit-bodies on a diploid mycelium.

A haploid fruit-body of *Coprinus lagopus*: (1) develops relatively late on its mycelium; (2) its pileus is pale owing to the development of but few or no spores on its gills; (3) its pileus often fails to expand properly; (4) it liberates but few or no spores; and (5) its spores, when produced, are all of one sex and of the same sex as that of the parent mycelium, *i.e.* (*AB*) or (*ab*) or (*Ab*) or (*aB*).

A diploid fruit-body of *Coprinus lagopus*, on the other hand: (1) develops relatively early on its mycelium; (2) its pileus turns

black owing to spores being developed all over its gills ; (3) its pileus always expands fully ; (4) it liberates a great many spores ; and (5) its spores, sexually, are of all the four possible kinds : (*AB*), (*ab*), (*Ab*), and (*aB*).¹

The relative earliness of fruiting in diploid mycelia of *Coprinus lagopus* as compared with haploid mycelia is illustrated in Fig. 139. The fourteen cultures (pairings of haploid mycelia) there shown were started on the same day. The nine which became diploidised have all produced fruit-bodies, while the five which remained haploid either have not yet begun to form fruit-bodies or their fruit-bodies are relatively less advanced than those in the diploid cultures.

There can be no doubt that, from the point of view of reproducing the species, it is advantageous for *Coprinus lagopus* to produce diploid fruit-bodies rather than haploid fruit-bodies. This being so, and since diploid fruit-bodies are produced only on diploid mycelia and haploid fruit-bodies only on haploid mycelia, it is far better for *C. lagopus* to develop its fruit-bodies on diploid mycelia than on haploid mycelia.

Since, in *Coprinus lagopus*, diploid mycelia produce much more effective fruit-bodies than haploid mycelia, it is important that the mycelium of the fungus present in a dung-ball should become diploidised to the greatest possible extent and particularly at the surface of the dung-ball where the fruit-body rudiments come into existence.

One of the means employed by *Coprinus lagopus* for increasing the amount of diploid mycelium in a dung-ball which has received many spores is the diploidisation of haploid mycelia by appropriate diploid mycelia, *i.e.* the diploidisation of (*AB*) and (*ab*) mycelia by (*AB*)+(*ab*) mycelia and the diploidisation of (*Ab*) and (*aB*) mycelia by (*Ab*)+(*aB*) mycelia.

As an illustration of the advantage accruing to *Coprinus lagopus*

¹ Presumably, in *Coprinus lagopus*, as in other Agaricineae, in a haploid fruit-body the cells contain isolated non-conjugate nuclei and each young basidium a single nucleus, while in a diploid fruit-body the cells contain conjugate nuclei and each young basidium a pair of conjugate nuclei which soon fuse together to form a fusion nucleus which undergoes reduction ; but I have not verified these suppositions by means of cytological observations.



FIG. 139.—*Coprinus lagopus*. To illustrate the fact that diploid mycelia fruit sooner than haploid mycelia. The fourteen Petri dishes containing dung-agar were inoculated on the same day with pairs of haploid mycelia, each mycelium derived from a single basidiospore. The photograph was taken about a week later. In the nine right-hand cultures in which mycelium No. 14 was paired with mycelia Nos. 18, 19, 20, 23, 25, 26, 27, 28, and 29, the two mycelia of each pair diploidised one another, as was shown by the development of clump-connections on their hyphae. In the five left-hand cultures in which mycelium No. 14 was paired with mycelia Nos. 15, 16, 17, 21, and 22, the two mycelia of each pair failed to diploidise one another and remained in the haploid condition, as was shown by their hyphae not developing any clump-connections. The nine diploid pairs of mycelia (on the right) have all produced diploid fruit-bodies: in eight of the dishes the fruit-bodies have expanded, deliquesced, and shed their spores; and in one dish (No. 14+No. 20) the fruit-body is a day behind the others (it expanded next day). On the other hand, in the five haploid pairs of mycelia (on the left), fruit-bodies either have not yet begun to develop or (as in No. 14+No. 17) are not far advanced. Fruit-bodies eventually appeared on all the five haploid pairs of mycelia, but they were haploid and—as usual with haploid fruit-bodies in *C. lagopus*—they were imperfect, so that they developed very few spores or no spores at all. Photographed by Irene Mounce. Reduced to one-third the natural size.

from its diploid mycelia being able to diploidise appropriate haploid mycelia, let us consider a case which, although extreme, brings out the point at issue. Let us suppose: (1) that, in a newly-dropped dung-ball, there are scattered fairly uniformly in a plane down the centre fifty spores of which the middle spore has the constitution (AB) and all the other spores the constitution (ab) ; (2) that all the spores germinate and produce mycelia which eventually make contacts with their nearest neighbours; and (3) that the haploid mycelium (AB) meets and pairs with a haploid mycelium (ab) with resultant mutual diploidisation and the formation of a diploid mycelium $(AB)+(ab)$ before this diploid mycelium comes into contact with any other mycelia. We know, on the basis of exact experiment, that the diploid mycelium $(AB)+(ab)$ on coming into contact with the other forty-eight haploid mycelia (ab) separately or as a net-work would diploidise them, and so cause diploid mycelium to be developed all around the outside of the dung-ball. Such a diploidisation of all the remaining forty-eight mycelia (ab) by the diploid mycelium $(AB)+(ab)$ would lead to the production of diploid instead of haploid fruit-bodies at the surface of the dung-ball, and so would facilitate the production of spores and therefore also the dissemination of the species. On the other hand, if the diploid mycelium $(AB)+(ab)$ were unable to diploidise the remaining forty-eight haploid mycelia, then, since the diploid mycelium $(AB)+(ab)$ had its origin at the centre of the dung-ball, most, and possibly all, of the network of mycelium developed at and near the surface of the dung-ball would be haploid, and this extensive haploid mycelium would give rise to nothing but imperfect and unprofitable haploid fruit-bodies. Thus, in the case under discussion and doubtless also extremely often under natural conditions, it is certain that the diploidisation of a haploid mycelium by an appropriate diploid mycelium is an advantageous phenomenon, in that it increases the number of spores which the fruit-bodies produce and so assists in maintaining the species in the environment in which it is fitted to live.

The Biological Significance of Conjugate Nuclei.—Let n represent the number of chromosomes in any nucleus and a pair of brackets () a nuclear membrane. Then a pair of conjugate nuclei

in the diploid mycelium of the Hymenomycetes, the Uredineae, the Ustilaginaceae, the Tilletiaceae, and the Exoascaceae and in the ascogenous hyphae of the Pyrenomycetes and the Discomycetes can be represented by the symbol $(n)+(n)$, while the single nucleus in the zygote and somatic or sporophytic cells of animals, Phanerogamia, Pteridophyta, and Bryophyta can be represented by the symbol $(2n)$.

What biological advantage, if any, is there in the diploid mycelium of the Basidiomycetes and the Exoascaceae and of the ascogenous hyphae of the Pyrenomycetes and the Discomycetes containing $(n)+(n)$ nuclei instead of $(2n)$ nuclei?

An attempt to answer this question will be made first for the Hymenomycetes and subsequently for the Uredineae, the Smut Fungi, the Exoascaceae, the Pyrenomycetes, and the Discomycetes.

The Hymenomycetes. Animals and most plants produce gametes. These special sex cells are characterised by being unicellular and by possessing a single nucleus containing n chromosomes; and when, during the fertilisation or conjugation process, two gametes of opposite sex meet and fuse, they form a single cell having a single nucleus containing $2n$ chromosomes.

The Hymenomycetes, in contrast with animals and most plants, have no sexual organs and never produce gametes; and yet they exhibit a very definite sexual process. Most of the species are heterothallic. A heterothallic species, in respect to sex factors,

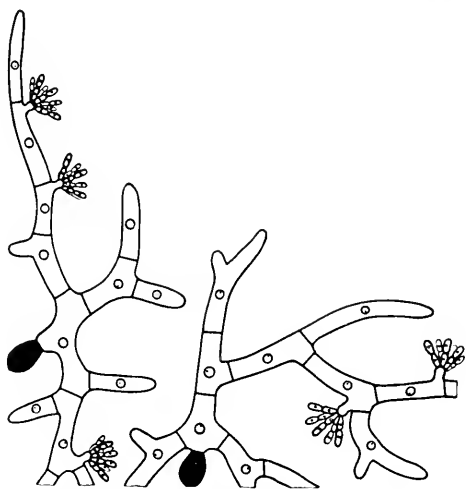


FIG. 140.—*Coprinus lagopus*. Diagram showing two haploid mycelia of the same sexual constitution (ab), each derived from a single basidiospore. Note the simple septa, the wide-angled mode of branching, the oidiophores with their groups of oidia, and the isolated nuclei. The two mycelia, with further growth, would soon fuse hyphally with one another; but, in the fusion cells, the nuclei would not become associated in conjugate pairs. Highly magnified.

may have only two kinds of spores, (*AB*) and (*ab*), as in *Coprinus Rostrupianus*¹ and *Fomes pinicola*,² or it may have four kinds of spores, (*AB*), (*ab*), (*Ab*), and (*aB*), as in *Coprinus lagopus*,³ *Armillaria mucida*,⁴ *Schizophyllum commune*,⁵ and *Aleurodiscus polygonius*.⁶

The spores of any heterothallic species are haploid

and give rise to haploid mycelia (Fig. 140), the nuclei of which we may suppose contain n chromosomes; and, when two haploid mycelia of opposite sex come into contact, they unite with one another by means of hyphal fusions and convert one another into diploid mycelia (Figs. 141, 142, and 145; also Plates I-II). The diploid mycelia thus produced always contain conjugate pairs of nuclei (n)+(n) and never isolated ($2n$) nuclei.

The number of pairs of conjugate nuclei originally formed in a hymenomycetous haploid mycelium during the

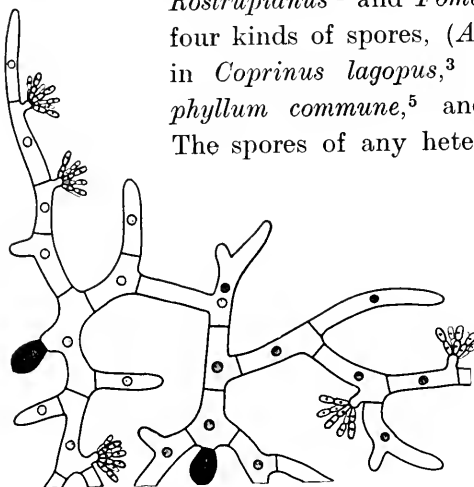


FIG. 141.—*Coprinus lagopus*. Diagram showing two haploid mycelia of opposite sex, (*AB*) on right (black nuclei), (*ab*) on left (white nuclei), each derived from a single basidiospore. Note the simple septa, the wide-angled mode of branching, the oidiophores with their groups of oidia, and the isolated nuclei. The two mycelia have just effected a hyphal fusion and, in the fusion cell, an (*AB*) nucleus and an (*ab*) nucleus have become associated as a pair of conjugate nuclei. The diploid cell is able to diploidise all the other cells of both mycelia by a process illustrated in Fig. 145. Highly magnified.

¹ Dorothy E. Newton, "The Bisexuality of Individual Strains of *Coprinus Rostrupianus*," *Annals of Botany*, vol. xl, 1926, pp. 105-128.

² Irene Mounce, "Studies in Forest Pathology. II. The Biology of *Fomes pinicola*," *Dominion of Canada, Dept. of Agric., Bull.* 111, 1929, pp. 1-74.

³ W. F. Hanna, "The Problem of Sex in *Coprinus lagopus*," *Annals of Botany*, vol. xxxix, 1925, pp. 431-457.

⁴ Hans Kniep, "Über morphologische und physiologische Geschlechtsdifferenzierung (Untersuchungen an Basidiomyceten)," *Verh. Physik.-mediz. Ges. Würzburg*, Bd. XLVI, 1920, pp. 1-18.

⁵ Hans Kniep, *ibid.*; also *Die Sexualität der niederen Pflanzen*, Jena, 1928, pp. 1-544.

⁶ Hans Kniep, "Über Geschlechtsbestimmung und Reduktionsteilung," *Verh. Physik.-mediz. Ges. Würzburg*, Bd. XLVII, 1922, pp. 1-28.

diploidisation process increases rapidly as the hyphae of the diploidised mycelium grow in length, owing to the fact that cell-division is preceded by conjugate nuclear division. In the

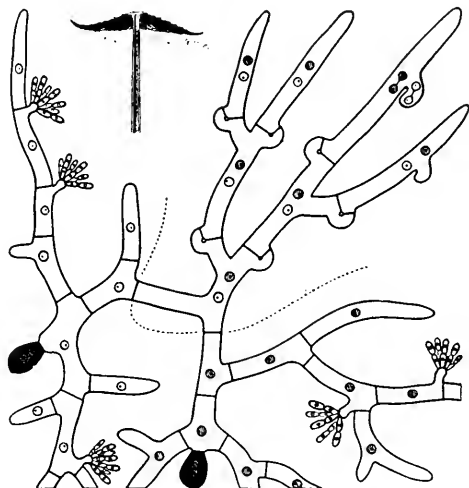


FIG. 142.—*Coprinus lagopus*. Diagram showing two haploid mycelia of opposite sex, (*AB*) on right (black nuclei), (*ab*) on left (white nuclei), each derived from a single basidiospore. The two mycelia, as shown in Fig. 141, fused hyphally and formed a fusion cell in which an (*AB*) nucleus and an (*ab*) nucleus became associated as a pair of conjugate nuclei. The fusion cell, as here shown, has elongated, branched, and developed into a diploid mycelium characterised by a narrow-angled mode of branching, by the presence of a pair of conjugate nuclei in every cell, by conjugate division of the pairs of nuclei accompanied by the formation of clamp-connexions, and by the absence of oidioophores and oidia. On the longest diploid hypha a conjugate nuclear division and the formation of a clamp-connexion is in progress. For the sake of simplicity, only one diploidised cell and the diploid mycelium into which it has grown has been represented; the other cells of the two original haploid mycelia have been left in the haploid state. In reality, the two haploid mycelia would have diploidised one another, and the diploidised terminal cells of all the hyphae of both originally haploid mycelia would have grown out into diploid mycelia like the one shown. The diploid mycelium produced by the mutual diploidisation of the two haploid mycelia would, in the end, produce diploid fruit-bodies, the upper half of one of which in vertical section is represented as discharging spores. Highly magnified.

diploid mycelium of very many Hymenomycetes, e.g. *Coprinus lagopus*, each cell-division is accompanied by the formation of a clamp-connexion between the two daughter cells (Figs. 85, p. 155, 142, and 146, p. 278). Hence a clamp-connexion is the outward and visible sign of the presence of conjugate nuclei in each of the two cells between which it lies. A diploid mycelium gives rise

to one or more diploid fruit-bodies; and, as a fruit-body develops, conjugate nuclear division continues. At last, a conjugate pair of nuclei becomes cut off in each basidium (Fig. 143, No. 1) and then conjugate nuclear division ceases. In each

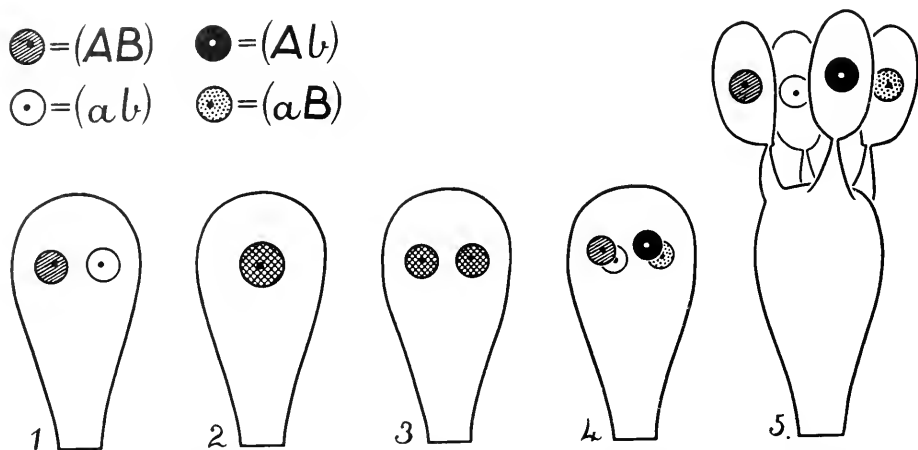


FIG. 143.—*Coprinus lagopus*. Diagram showing the completion of the sexual process in a basidium, including the fusion of the two members of a conjugate pair of nuclei, the two successive divisions of the fusion nucleus, and the formation of four haploid nuclei, one of which enters each of the four spores. The sexual constitution of each haploid nucleus is indicated by shading in accordance with the scheme shown on the upper left. No. 1: a young basidium containing a pair of conjugate nuclei (AB) and (ab). No. 2: the (AB) and the (ab) nuclei have fused; the diploid nature of the fusion nucleus is indicated by cross-hatching. No. 3: the fusion nucleus has undergone its first division; the daughter nuclei, like their parent, are diploid, as indicated by the cross-hatching. No. 4: the two daughter nuclei of the fusion nucleus have divided; this second division of the fusion nucleus was accompanied by the reduction of the chromosomes to one-half their number (presumably), and by the segregation of the sex factors; the four resultant nuclei here represented are all different from one another and have the sexual constitution (AB), (ab), (Ab), and (aB) respectively; but it is a matter of chance whether in any particular basidium the four nuclei belong to any one of the three following groups: (AB), (ab), (Ab), (aB); (AB), (AB), (ab), (ab); and (Ab), (Ab), (aB), (aB). (Cf. Fig. 144.) No. 5: the basidium-body has developed four sterigmata and four basidiospores, and the four haploid nuclei have crept through the narrow necks of the sterigmata into the spores. Thus each spore is a haploid cell, and, in the instance represented, the four spores have the sexual constitution (AB), (ab), (Ab), and (aB) respectively. Highly magnified.

young basidium the two nuclei of opposite sex unite, and the fusion nucleus so produced soon undergoes two successive divisions accompanied by a reduction in the number of chromosomes to one-half and the segregation of sex and other factors (Fig. 143, Nos. 2-4). Four sterigmata and four spores now develop on each

basidium. Finally, a nucleus creeps from the basidium-body through a sterigma into each spore, so that, at last, each spore

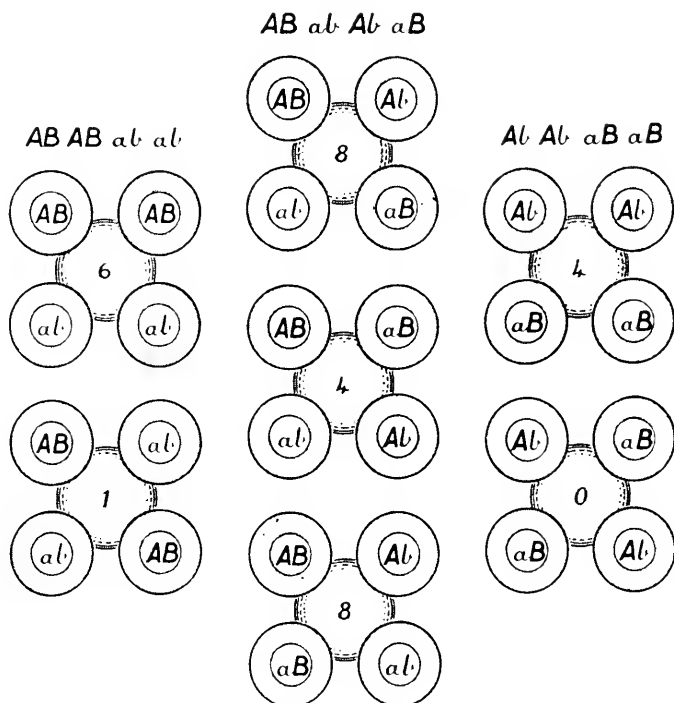


FIG. 144.—*Coprinus lagopus*. Diagram showing the arrangement of the spores of diverse sex on thirty-one basidia seen from above. The four kinds of spores (AB), (ab), (Ab), and (aB) can be arranged on a basidium in seven different ways, all of which are here represented. The inner circle in each spore represents a nucleus, and the symbols within a pair of sex factors. The number in the centre of each basidium gives the actual number of basidia (of the thirty-one investigated) found with the arrangement of spores represented. The first column shows the two possible arrangements when there are two (AB) and two (ab) spores on a basidium; the second column the three possible arrangements when there are four kinds of spores, (AB), (ab), (Ab), and (aB), on a basidium; and the third column the two possible arrangements when there are two (Ab) and two (aB) spores on a basidium. Investigations carried out by Dorothy Newton. Drawn by the author and Dorothy Newton.

becomes a haploid cell (Fig. 143, No. 5). In *Coprinus lagopus*,¹ as shown in Fig. 144, there are three kinds of basidia. (1) basidia which bear two (AB) and two (ab) spores; (2) basidia which bear

¹ Dorothy E. Newton, "The Distribution of Spores of Diverse Sex on the Hymenium of *Coprinus lagopus*," *Annals of Botany*, vol. xl, 1926, pp. 891-917.

two (*Ab*) and two (*aB*) spores; and (3) basidia which bear spores of all four possible kinds (*AB*), (*ab*), (*Ab*), and (*aB*). In *Coprinus Rostrupianus*¹ there is only one kind of basidium, and this bears two (*A*) spores and two (*a*) spores.

In animals and most plants, when two gametes unite, a ($2n$) nucleus is formed in the zygote. Why then, in Hymenomycetes, when two haploid mycelia of opposite sex unite, are not ($2n$) nuclei formed instead of conjugate pairs of nuclei (n)+(n)? Why does such a union of haploid mycelia result at first only in nuclear association followed by a long series of conjugate nuclear divisions, so that there is a delay in nuclear fusion until the basidia are formed?

The production of ($2n$) nuclei when two animal or vegetable gametes of opposite sex unite is correlated with the fact that every gamete is a *single cell* and contains a *single nucleus*; whereas, the production of (n)+(n) nuclei when two hymenomycetous haploid mycelia of opposite sex unite is correlated with the fact that the two mycelia, when the sexual process is initiated, are usually *multicellular* and *multinuclear*.

As we have seen, a tiny mass of hyphae of a haploid mycelium, after being set at the periphery of another mycelium of opposite sex 6·0 cm. in diameter, is able to diploidise the large mycelium in the course of about three days. In such a physically unequal combination as the one under consideration, it is obvious that the nuclei of the large mycelium which receive conjugate mates during the diploidisation process must be more than one hundred times as numerous as the nuclei contained in all the hyphae of the tiny fragment of mycelium with which the large mycelium was inoculated. It is easily conceivable that a large haploid mycelium 6·0 cm. in diameter might be diploidised by a *single nucleus* entering it from a mycelium of opposite sex.

When two animal or plant gametes unite and form a zygote, the two gametes contribute each the *same number* of nuclei—one—to the formation of the zygote; whereas, when two hymenomycetous haploid mycelia of opposite sex unite and diploidise one

¹ Dorothy E. Newton, "The Bisexuality of Individual Strains of *Coprinus Rostrupianus*," *ibid.*, pp. 105-128.

another, each mycelium receives from its mate not a number of nuclei equal to the number of its own nuclei, but relatively *very few nuclei* (sometimes, perhaps, only one).

In order to understand how it is that one or a few nuclei derived from one haploid mycelium are able to bring about the diploidisation of another large haploid mycelium of opposite sex, it will be necessary to inquire into the details of the diploidisation process and to find out exactly what part conjugate pairs of nuclei play therein.

Relying on the cytological work of Lehfeldt (to which reference has already been made, p. 216) and on my own observations on the direct transformation of haploid hyphae into diploid hyphae as indicated by the production of clamp-connexions and by a change from the haploid to the diploid mode of branching (*vide* Fig. 133, p. 238), let us endeavour to imagine the course of the diploidisation process in a large haploid mycelium of *Coprinus lagopus*, containing 100,000 (*ab*) nuclei scattered in its hyphae, after the mycelium has met with, and fused with, a single hypha of a mycelium of opposite sex and has received through the hypha a single (*AB*) nucleus.

The (*AB*) nucleus, on entering the (*ab*) mycelium, at once begins to divide and subdivide, and the (*AB*) nuclei so produced move through the three-dimensional network of hyphae which make up the (*ab*) mycelium. Soon, certain of the (*AB*) nuclei associate themselves individually with certain (*ab*) nuclei and a number of conjugate pairs of nuclei (*AB*)+(*ab*) become established, particularly at the ends of the rapidly-growing hyphae in the neighbourhood of the (*AB*) hypha. To allow of the passage of the (*AB*) nuclei through the (*ab*) network of hyphae, the simple septa of the (*ab*) hyphae become partially or wholly broken down as (*AB*) nuclei approach them. Whilst some of the (*AB*) nuclei form conjugate pairs with certain of the (*ab*) nuclei, other (*AB*) nuclei are moving through the (*ab*) mycelium away from the (*AB*) hypha and away from the (*ab*) hyphae which already have come to contain conjugate pairs of nuclei. These moving (*AB*) nuclei divide and subdivide and some of them are constantly settling down in particular cells, especially the younger ones, where they form conjugate

mates for the (*ab*) nuclei there present. Thus, proceeding away from the (*AB*) hypha, the number of (*ab*) hyphae which become diploidised is progressively increased.

In the terminal cell of any elongating hypha, in which a pair of conjugate nuclei has become established, cell-division accompanied by conjugate nuclear division and the formation of clamp-connections soon sets in, and thus the number of pairs of conjugate nuclei becomes increased.

Let us now suppose that an (*AB*) nucleus makes its way, *via* a bridging hypha, into the middle cell of three cells of a single (*ab*) hypha (Fig. 145, Nos. 1-3). We may suppose that the (*AB*) nucleus becomes conjugate with the (*ab*) nucleus. Then we have three cells in a row, the middle cell containing a conjugate pair of nuclei (*AB*)+(*ab*) and each of the end-cells an (*ab*) nucleus. The diploidisation of these two end-cells becomes effected very simply (Fig. 145, Nos. 4-6). The (*AB*) nucleus of the middle cell divides and so liberates an (*AB*) nucleus which travels into one of the end-cells where it becomes associated with the (*ab*) nucleus there present. Then the (*AB*) nucleus of the middle cell divides again and so liberates a second (*AB*) nucleus which travels into the other end-cell where it becomes associated with the (*ab*) nucleus there present. Thus the middle cell has rapidly diploidised its two neighbouring cells by a very simple process.

Let us suppose that a diploid lateral branch of a diploidised (*ab*) hypha meets and fuses with a non-terminal cell of a radial or other (*ab*) hypha which is still in the haploid condition (*cf.* Fig. 148, Nos. 1-3). Then we have three nuclei to consider, a conjugate pair (*AB*)+(*ab*) in the end cell of the diploid branch-hypha and a single nucleus (*ab*) in the non-terminal cell of the haploid hypha. The diploidisation of the cell of the haploid hypha is rapidly effected by the same process as that described in the last paragraph: the (*AB*) nucleus of the conjugate pair (*AB*)+(*ab*) divides, and thus provides an additional (*AB*) nucleus which moves into the haploid cell and becomes associated with the (*ab*) nucleus there present. Again, a diploid cell has diploidised a neighbouring haploid cell.

In general, as the (*ab*) mycelium becomes diploidised, wherever

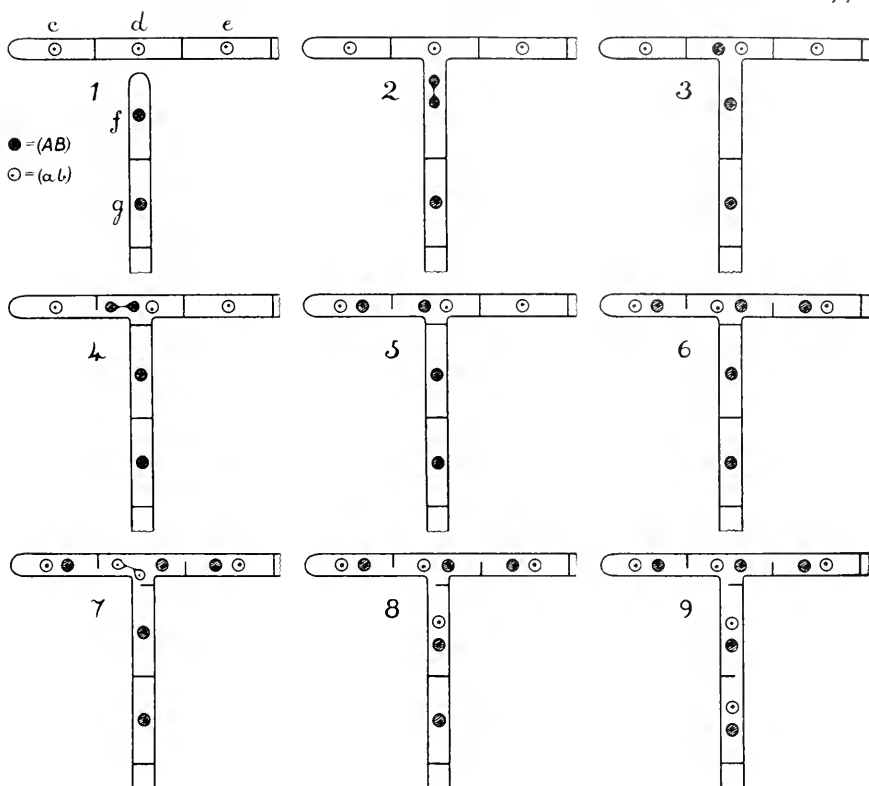


FIG. 145.—*Coprinus lagopus*. Diagram to illustrate the mutual diploidisation of two haploid mycelia of opposite sex. It has been assumed that all the cells of both of the hyphae shown become diploidised, that nuclei may pass through a hyphal fusion in both directions, and that the septa break down in turn as required for the passage of nuclei from one cell to another. No. 1: a hypha $f\ g$ of a haploid mycelium (AB) is growing toward, and soon will meet and fuse with, the cell d of the hypha $c\ d\ e$ of a haploid mycelium (ab). No. 2: fusion has taken place, and the (AB) nucleus of f is dividing. No. 3: one of the daughter (AB) nuclei has passed into the cell d , thus diploidising it. No. 4: a wall now separates the cells d and f , and the (AB) nucleus in the cell d is dividing. No. 5: the wall between the cells c and d is partly broken down, and one of the daughter (AB) nuclei has passed through it from d to c . No. 6: the (AB) nucleus of the cell d has again divided and has sent one of its daughter (AB) nuclei into the cell e . Thus the haploid cell f has diploidised the haploid cell d , and the diploid cell d in its turn has diploidised first the haploid cell c and then the haploid cell e . Nos. 7, 8, and 9 show the diploidisation of the cells f and g of the (AB) hypha by means of nuclei derived from the formerly haploid mycelium (ab). No. 7: the (ab) nucleus of the conjugate pair of nuclei (AB) and (ab) in the cell d is now dividing. No. 8: the wall between the cells d and f is partly broken down, and one of the daughter (ab) nuclei has passed through it from d to f . No. 9: the (ab) nucleus of the cell f has divided and has sent one of its daughter (ab) nuclei into the cell g . Thus the diploidisation of all the cells shown in the two hyphae has been accomplished. The further development of a diploidised hypha including the conjugate division of the conjugate pairs of nuclei and the formation of clamp-connexions is represented diagrammatically in Fig. 146. Highly magnified.

a pair of conjugate nuclei $(AB)+(ab)$ becomes established in a cell next to another cell, either older or younger, which contains only an (ab) nucleus and therefore is still haploid, the (AB) nucleus

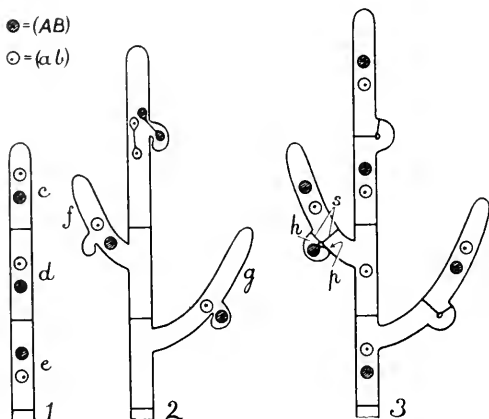


FIG. 146.—*Coprinus lagopus*. Diagram to show how a haploid hypha after it has just been diploidised develops further. No. 1: a haploid hypha (ab) or (AB) which has just been converted into a diploid hypha $(AB)+(ab)$; a pair of conjugate nuclei is present in each cell. No. 2: the cells d and e have branched and the cell c has elongated; in f a hook has grown backwards; in g an (AB) nucleus has passed into the hook; in the terminal cell c the two nuclei are dividing conjugately and the hook is growing toward the main hypha. No. 3: in the branch f the two daughter pairs of conjugate nuclei have separated from one another and two septa s have been formed, but the lower (AB) nucleus is momentarily a prisoner in the hook-cell h from which it will escape as soon as the walls at p have broken down; in the branch g and the terminal cell c conjugate nuclear division and cell-division with the formation of a clamp-connexion have been completed, and each daughter-cell contains a pair of conjugate nuclei (AB) and (ab) . Highly magnified.

of the conjugate pair divides and one of the daughter (AB) nuclei wanders into the haploid cell, associates itself with the (ab) nucleus there present, and so takes part in the formation of a second conjugate pair of nuclei $(AB)+(ab)$.

Thus, through the agency of a single initial (AB) nucleus, our large (ab) mycelium, which when it fused with the (AB) hypha contained 100,000 nuclei, becomes diploidised as a whole.

Assuming that the essential features of the diploidisation process as it goes on in a hymenomycetous haploid mycelium have been correctly indicated in the description of the conversion of a large haploid mycelium (ab) into a diploid mycelium $(AB)+(ab)$ as just

given, we must conclude that, in general, the diploidisation process in the Hymenomycetes is dependent on the formation of conjugate nuclei $(n)+(n)$ and on the avoidance of the formation of isolated $(2n)$ nuclei. The formation of conjugate pairs of nuclei permits one of each pair to divide independently of its fellow

whenever such a division is required to provide an extra nucleus needed in an adjacent haploid cell for the establishment of a second pair of conjugate nuclei. On the other hand, if two nuclei of opposite sex, on coming to be present in one and the same cell, were to fuse together to form a $(2n)$ nucleus, the $(2n)$ nucleus could do nothing to convert an (n) nucleus in an adjacent haploid cell into a $(2n)$ nucleus unless it were to undergo reduction divisions and produce among its progeny a nucleus of the right kind to mate with the (n) nucleus in the haploid cell. Obviously it is far simpler for a pair of conjugate nuclei $(n)+(n)$ to provide the (n) mate required by an (n) nucleus in an adjacent haploid cell than it would be for a $(2n)$ nucleus to do so.

The diploidisation of a haploid mycelium by a diploid mycelium in *Hymenomyces* like *Coprinus lagopus* (Figs. 147 and 148) is effected essentially in the same manner as the diploidisation of a haploid mycelium by another haploid mycelium of opposite sex. Thus, in the combination $(ab) \times (AB)+(ab)$, when a hypha of the (ab) mycelium fuses with a hypha of the $(AB)+(ab)$ mycelium, the (AB) nucleus in the conjugate pair of nuclei in the diploid hypha divides and one of the daughter nuclei moves into the hypha of the (ab) mycelium. The gradual diploidisation of the (ab) mycelium then proceeds in exactly the same manner as that described in the case of the combination of two haploid mycelia $(AB) \times (ab)$.

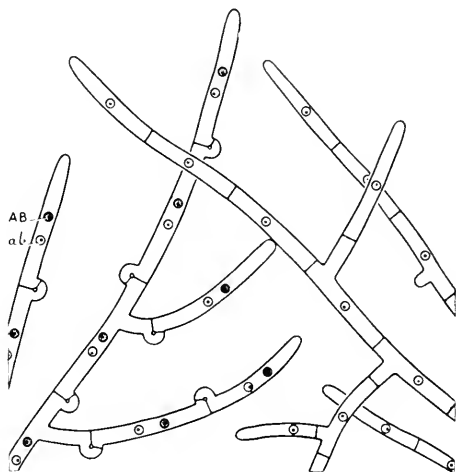


FIG. 147.—*Coprinus lagopus*. Diagram showing a diploid mycelium $(AB)+(ab)$ on the left, and a haploid mycelium (ab) on the right. In the diploid mycelium note the pairs of nuclei of opposite sex in each cell, the clamp-connexions at each septum, and the narrow-angled mode of branching. In the haploid mycelium note the single isolated nucleus in each cell, the plain septa devoid of clamp-connexions, and the wide-angled mode of branching. A central hypha of the diploid mycelium is about to fuse with a hypha of the haploid mycelium. The diploid mycelium, after the fusion was effected, would begin to diploidise the haploid mycelium (*vide* Plates III and IV). Highly magnified.

The complete diploidisation of any multicellular and multinuclear hymenomycetous haploid mycelium through the agency of a single nucleus or relatively few nuclei derived from a haploid mycelium of opposite sex or from a diploid mycelium containing

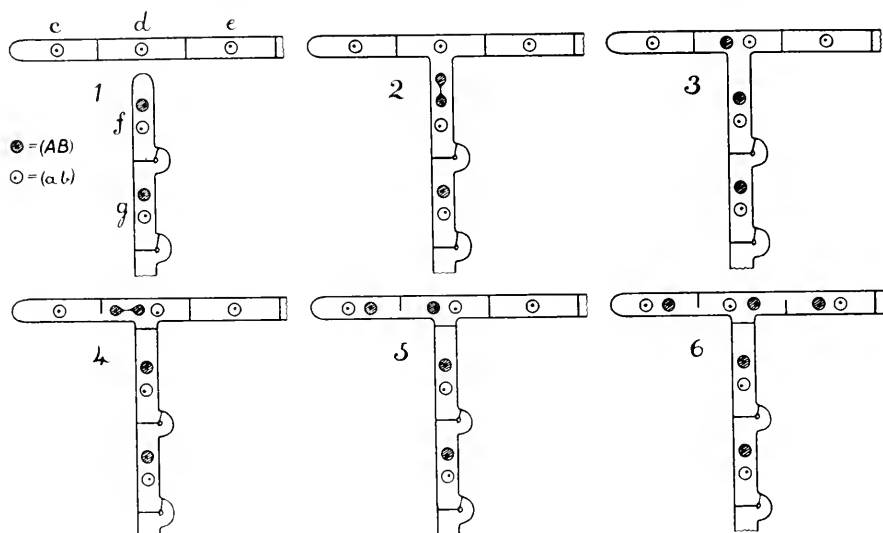


FIG. 148.—*Coprinus lugopus*. Diagram to show the diploidisation of a haploid mycelium (*ab*) by a diploid mycelium (*AB*)+(ab). No. 1: the cell *f* of the diploid hypha *fg* is growing toward, and soon will meet, and fuse with, the cell *d* of the haploid hypha *cde*. No. 2: fusion has taken place and the (*AB*) nucleus of *f* is dividing. No. 3: one of the daughter (*AB*) nuclei has passed into the cell *d*, thus diploidising it. No. 4: a wall now separates the cells *d* and *f*, and the (*AB*) nucleus in the cell *d* is dividing. No. 5: the wall between the cells *c* and *d* is partly broken down, and one of the daughter (*AB*) nuclei has passed through it from *d* to *c*. No. 6: the (*AB*) nucleus of the cell *d* has again divided and has sent one of its daughter (*AB*) nuclei into the cell *c*. Thus the diploid cell *f* diploidised the haploid cell *d* and the diploid cell *d* in its turn diploidised first the haploid cell *c* and then the haploid cell *e*. Highly magnified.

nuclei of opposite sex is absolutely dependent on the formation of conjugate nuclei.

The organisation of the nuclei in conjugate pairs (n)+(n) instead of as isolated nuclei ($2n$) in the diploid mycelium and fruit-body of the Hymenomycetes results, it is true, in delaying the fusion of nuclei of opposite sex until the basidia come into existence, but it has the great advantage that, in the diploid mycelium, each member of a pair of conjugate nuclei retains its identity, so that one member of a pair can divide independently of the other member of the pair

whenever such a division is able to promote the diploidisation of a haploid mycelium.

The Uredineae. The Rust Fungi, a group of highly specialised parasites, have probably been evolved from the Hymenomycetes which they resemble : (1) in producing basidia and basidiospores, (2) in the mode of development and discharge of their basidiospores, (3) in the absence of sexual organs and gametes, and (4) in the general nature of their sexual process.

The sexual process in a heterothallic Rust Fungus, such as *Puccinia graminis* or *P. helianthi*, when initiated by two haploid mycelia of opposite sex derived from two basidiospores of opposite sex, resembles that of the Hymenomycetes already described ; for it takes place as follows. Two haploid mycelia of opposite sex derived from two basidiospores (sporidia) of opposite sex meet within a host-leaf, unite with one another, and mutually diploidise one another so that, in the spore-bed of each aecidium, there are produced basal cells each of which contains a pair of conjugate nuclei.¹ As soon as the diploidisation process has been completed, cell-division preceded by conjugate nuclear division sets in and continues during the production of the aecidiospores, the development of the mycelium derived from an aecidiospore, the production of uredospores, the development of the mycelium derived from a uredospore, and the production of teleutospores. As soon as the teleutospores have been formed, conjugate nuclear division comes to an end and the two nuclei of the conjugate pair in each cell of each teleutospore fuse together. In the basidium (promycelium) developed from each cell of a teleutospore the fusion nucleus undergoes two successive divisions accompanied by a reduction in the number of chromosomes to one-half and a segregation of the sex and other heritable factors. Finally, the basidium gives rise to four sterigmata and four spores and each of the four (n) nuclei creeps through a sterigma and so enters a spore. Thus each basidiospore is a haploid cell.

If, as we have seen, conjugate nuclei are essential to the working of the diploidisation process as it occurs in a haploid mycelium of one of the Hymenomycetes, then, in all probability,

¹ J. H. Craigie, "Experiments on Sex in Rust Fungi," *Nature*, July, 1927.

it is essential to the working of the diploidisation process as it occurs in a haploid mycelium of a Rust Fungus.

From the foregoing it is clear that, in the Uredineae, during the diploidisation process, the nuclei of opposite sex do not unite with one another to form $(2n)$ nuclei, but retain their identity and eventually become associated with one another as conjugate nuclei $(n)+(n)$ in the basal cells of the spore-bed of each young aecidium.

Before it is possible to decide what biological advantage, if any, accrues to the Uredineae by the avoidance of the formation of $(2n)$ nuclei and the eventual arrangement of the nuclei in conjugate pairs $(n)+(n)$ during the diploidisation process, it is necessary to form some definite conception of the precise way in which the diploidisation process is effected; and this will now be attempted.

Let us suppose that two basidiospores (sporidia) of opposite sex, (A) and (a) , of some heterothallic long-cycle Rust Fungus have settled on a host-leaf near one another and have germinated, so that two young haploid mycelia of opposite sex are present in the leaf. Each haploid mycelium forms a rust pustule. As growth continues, the two mycelia come into contact with one another and the two rust pustules coalesce. How do these two mycelia, on coming into contact with one another, interact so as to form conjugate nuclei in the basal cells of the aecidia? In other words: how does the diploidisation process work? There are various possible answers to this question and two of them will now be brought forward and discussed.

Hypothesis No. 1. The two haploid mycelia (A) and (a) , as their growth proceeds, *intermingle* so that, eventually, two kinds of hyphae (A) and (a) come to be arranged almost alternately in the spore-bed of each young aecidium. Then, in the spore-bed, pairs of (A) and (a) cells combine to form conjugate nuclei: either, as in *Phragmidium violaceum*¹ or *Puccinia poarum*² by a cell-wall becoming perforated to allow of an (A) nucleus migrating into an (a) cell or *vice versa*, or, as in a number of other rust species, by

¹ V. H. Blackman, "On the Fertilization, Alternation of Generations, and General Cytology of the Uredineae," *Annals of Botany*, vol. xviii, 1904, pp. 323-373.

² V. H. Blackman and H. C. I. Fraser, "Further Studies on the Sexuality of the Uredineae," *Annals of Botany*, vol. xx, 1906, pp. 35-48.

the wall between an (*A*) and an (*a*) cell breaking down so that the (*A*) and (*a*) cells combine to form a single cell.

In support of Hypothesis No. 1, one may cite the illustrations of two cells in a spore-bed uniting laterally to form a single binucleate basal cell, as given among others by Christman¹ for *Phragmidium speciosum* and Colley² for *Cronartium ribicola*.

Hypothesis No. 2. The two haploid mycelia (*A*) and (*a*), on

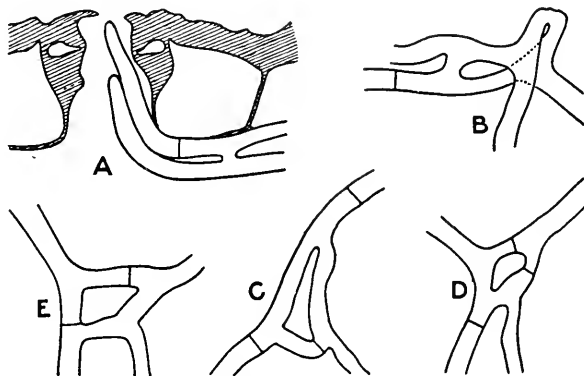


FIG. 149.—Hyphal fusions on the same mycelium in various species of Uredineae. A, *Puccinia* on *Carex hirta*; a bridging hypha uniting two hyphae in the aecidial mycelium. B, *Puccinia* on *Phragmites communis*; C, *Puccinia* on *Carex acuta*; and D, *Melampsora* on *Salix viminalis*: meshes in the aecidial mycelium. E, *Puccinia graminis*; a mesh in the uredinial mycelium. The drawings copied by the author from W. Voss ("Über Schnallen und Fusionen bei den Uredineen," *Ber. d. Deutsch. Bot. Gesell.*, Bd. XXI, 1903, Taf. XIX, Figs. 1, 4, 5, 8, and 11) and rearranged; shading added to the walls of the epidermal cells in A. Highly magnified.

coming into contact, *do not intermingle appreciably*, but each remains in the leaf-territory which it has grown through and more or less exhausted. Hyphal fusions take place between the two mycelia (*cf.* Fig. 149), with the result that one or more (*A*) nuclei move into the (*a*) mycelium and one or more (*a*) nuclei move into the (*A*) mycelium. *The (*A*) nuclei move along the hyphae of the*

¹ A. H. Christman, "Sexual Reproduction in the Rusts," *Botanical Gazette*, vol. xxxix, 1905, pp. 267-275.

² R. H. Colley, "Parasitism, Morphology, and Cytology of *Cronartium ribicola*," *Journ. Agric. Research*, vol. xv, 1918, pp. 619-660.

(*A*) mycelium and the (*a*) nuclei move along the hyphae of the (*A*) mycelium, and the moving nuclei undergo division and make their way to the places where the rudiments of the aecidia are being formed; so that, in the hyphae which form the aecidial rudiments, both (*A*) and (*a*) nuclei are present. These hyphae now form a series of uninucleate cells, some of which contain an (*A*) nucleus and some an (*a*) nucleus. Then the co-operation of (*A*) and (*a*) cells by means of nuclear migration or cell fusion sets in, and this leads to the formation in each aecidium of a spore-bed in which the basal cells contain conjugate nuclei (*A*)+(*a*).

In Hypothesis No. 2, the assumption that the (*A*) and (*a*) mycelia do not mingle appreciably in each other's original pustules is based on the fact that, in the Hymenomycetes, when two haploid mycelia of opposite sex are placed near together on a nutrient medium, on meeting with one another they do not intermingle appreciably. The non-intermingling of the (*A*) and (*a*) mycelia may be attributed to the exhaustion of the host-leaf locally by both the (*A*) and the (*a*) mycelia; so that (*A*) hyphae, on coming into contact with the (*a*) mycelium, cease to grow in length and (*a*) hyphae, on coming into contact with the (*A*) mycelium, also cease to grow in length.

The following assumptions of Hypothesis No. 2 are based on what is known of the diploidisation process in the Hymenomycetes: (1) that the (*A*) and the (*a*) mycelia, on coming into contact with one another, unite by means of one or more hyphal fusions; (2) that one or more (*A*) nuclei move through one or more hyphal bridges into the (*a*) mycelium where they divide and find their way to the localities where the (*a*) mycelium is forming aecidial rudiments; and (3) that one or more (*a*) nuclei move through one or more hyphal bridges into the (*A*) mycelium where they divide and find their way to the localities where the (*A*) mycelium is forming aecidial rudiments.

The assumption in Hypothesis No. 2 that the hyphae which form the rudiments of the aecidia at first contain both (*A*) and (*a*) nuclei, but subsequently undergo cell-division so as to produce a mass of uninucleate cells of which some contain an (*A*) nucleus and others an (*a*) nucleus, has been made in order to account for

the mixed arrangement of uninucleate (A) and (a) cells in the young aecidium at the moment when the (A) and (a) cells there present co-operate in pairs to form basal cells containing conjugate nuclei.

Of the two hypotheses suggested above I am inclined to favour the second because, in comparison with the first, it seems simpler and more in accordance with the process of diploidisation as known in the Hymenomycetes.

The most important part of Hypothesis No. 2 seems to me to be the assumption that, in the Uredineae, one or a few nuclei derived from a haploid mycelium of one sex can move into and through a haploid mycelium of opposite sex, multiply in number there, and so provide one member of each conjugate pair of nuclei in each basal cell of the spore-bed of every aecidium which the mycelium produces. The details of the process by which the two kinds of nuclei come to be assembled as conjugate pairs in the basal cells may possibly vary in different species, in which case the final part of the hypothesis might require modification.

Assuming that, when two haploid mycelia (A) and (a) of the same Rust species meet, they unite and that, subsequently, (A) nuclei move through the (a) mycelium and (a) nuclei through the (A) mycelium, it is clear (1) that the septa must break down partially or wholly (as in Hymenomycetes) to permit of the progress of the nuclei and (2) that two nuclei of opposite sex, when they meet in one and the same mycelium, are inhibited from fusing with one another. The assumption of such an inhibition accounts for the fact that the nuclei of opposite sex assembled in the basal cells of each aecidium as conjugate nuclei do not fuse with one another but divide conjugately.

Assuming that the essential features of the diploidisation process as it goes on in a multicellular and multinuclear haploid mycelium of a Rust Fungus has been correctly indicated in Hypothesis No. 2, we are justified in concluding that the diploidisation process in the Uredineae, just as in the Hymenomycetes, is dependent on the avoidance of the formation of ($2n$) nuclei. In the Uredineae this results in the free association of nuclei of one sex with those of the other sex in the mycelium becoming diploidised and, finally, in

the association of the nuclei in conjugate pairs in the basal cells of the spore-bed of each aecidium.

The diploidisation process in a haploid mycelium of a Rust Fungus such as *Puccinia graminis* and *P. helianthi* can be initiated not only (1) by another mycelium of opposite sex but, also, as Craigie¹ has shown, (2) by means of pycnidiospores applied to the mouths of pycnidia produced on the haploid mycelium; but, however it originates, its essential features are doubtless always the same. Possibly, as Miss Allen's work on *Puccinia graminis* suggests,² the pycnidiospores put out germ-tubes which fuse with the paraphyses (ostiole hairs of a pycnidium) or other cells of the mycelium already in the leaf and deliver to this mycelium nuclei which travel down through the hyphae, divide, and find their way to the spore-bed of each rudimentary aecidium where, by further division and migration, they diploidise every cell that is destined to produce a chain of aecidiospores.³

¹ J. H. Craigie, "Discovery of the Function of the Pycnia of the Rust Fungi," *Nature*, November, 1927.

² Ruth F. Allen, "A Cytological Study of Heterothallism in *Puccinia graminis*," *Journ. Agric. Research*, vol. xl, 1930, pp. 585-614.

³ It is possible that in the Uredineae, just as in the Hymenomycetes, there are included not only heterothallic species, but also homothallic. Among the Rust species which I suspect are homothallic are short-cycle species devoid of pycnidia, e.g. *Puccinia Malvacearum*. This species is entirely dependent upon sporidia for its dissemination. In a garden at Kew, in the summer of 1930, on searching the Hollyhocks (*Althaea rosea*), I found that they were entirely free from *P. Malvacearum*, except for one leaf on which was a solitary pustule bearing the usual chocolate-brown teleutospores. It seems unlikely that this solitary pustule should have arisen from two sporidia of opposite sex which happened to have been blown into the garden and to have settled on the leaf in exactly the same spot, and the pustule could not have arisen from a single haploid sporidium and then have been diploidised by pycnidiospores brought by insects from a distance, for *P. Malvacearum* has no pycnidia. In the heterothallic Coprini oidia are generally present on the haploid mycelia, but in the homothallic species, *Coprinus stercorearius*, *C. sterquilinus*, and *C. narcoticus*, they are absent. From the investigations made by H. J. Brodie, working under my direction ("The Oidia of *Coprinus lagopus* and their Relation with Insects"; to appear in the *Annals of Botany*, April, 1931), there is every reason to believe that the oidia of the heterothallic Coprini and the pycnidiospores of the heterothallic Uredineae are comparable in their diploidising function. In a homothallic Coprinus oidia would be useless, and in a homothallic *Puccinia* pycnidiospores would be equally useless. Perhaps it is on account of the early setting in of the diploid phase in mycelia of monobasidiosporous origin that oidia and pycnidiospores have ceased to be developed in homothallic Coprini and the presumably homothallic *Puccinia Malvacearum* respectively.

If Hypothesis No. 2 is a sound one, then the biological advantage which accrues to the Uredineae in having the nuclei in their diploid mycelium organised as conjugate pairs of nuclei $(n)+(n)$ rather than as isolated nuclei $(2n)$ lies in this: that the nuclear arrangement just indicated is correlated with an inhibition which prevents two nuclei of opposite sex fusing with one another, which inhibition makes possible the diploidisation of an extensive multicellular and multinuclear haploid mycelium by means of one or a few nuclei derived either from another haploid mycelium of opposite sex or from one or more pycnidiospores of opposite sex.

The Ustilaginaceae and the Tilletiaceae. The Smut Fungi, like the Rust Fungi, are a group of highly specialised parasites; and it may be that they have been evolved from some branch of the Hymenomycetes. In any case, there can be but little doubt that the Hymenomycetes, the Rust Fungi, and the Smut Fungi have all been derived from a common basidiomycetous stock.

The Smut Fungi, like all other Basidiomycetes, are devoid of sexual organs and of true gametes; and their sexual process, in its general features, resembles that of the Hymenomycetes and the Rust Fungi. Union takes place between two morphologically indistinguishable sporidia outside the host (*Ustilago violacea*, *U. avenae*), or between two haploid mycelia inside the host (*Ustilago zaeae*), and a diploid mycelium containing conjugate nuclei is then formed. This mycelium develops in the host plant; and, as it grows and forms new cells, conjugate nuclear division is accompanied by the formation of clamp-connexions (*Ustilago*, *Doassansia*, *Urocystis*, *Tilletia*, *Entyloma*, *Tubercinia*).¹ A pair of conjugate nuclei is cut off in each young chlamydospore and then conjugate nuclear division comes to an end. The two nuclei of the pair soon unite, so that the mature chlamydospore is uninucleate. When a chlamydospore germinates, the fusion nucleus $(2n)$ divides twice, or in some cases more times, and these nuclear divisions are accompanied by a segregation of the sex factors and doubtless also by a reduction in the number of chromosomes to one-half. Each haploid nucleus (n) finds its way into a sporidium, and so haploid cells are once more brought into existence.

¹ R. Seyfert, "Über Schnallenbildung im Paarkernmyzel der Brandpilze," *Zeitschrift f. Botanik*, Bd. XIX, 1927, pp. 577-601.

In *Ustilago violacea* and *U. avenae*, according to Kniep,¹ it is not possible to infect the host plant with unisexual cultures of sporidia, whilst infection is readily effected with cultures containing sporidia of both sexes. Apparently, two sporidia of opposite sex unite on the surface of the host and then send a diploid mycelium into the host's interior. It would therefore appear that, in *U. violacea* and *U. avenae*, a diploidisation of a multinucleate haploid mycelium of one sex by one or a few nuclei derived from another haploid mycelium of opposite sex does not occur.

On the other hand, in *Ustilago zeae*, according to Hanna,² the host plant becomes infected with haploid mycelia, and two haploid mycelia of opposite sex, on meeting inside the host, fuse together and give rise to a diploid mycelium. It may be that, in this species, as in the Hymenomycetes, owing to the formation of conjugate nuclei $(n)+(n)$ instead of $(2n)$ nuclei, one or a few nuclei of a haploid mycelium of one sex are able to diploidise all the rapidly-growing hyphae of another haploid mycelium of opposite sex. If diploidisation is able to proceed in this way, the avoidance of the formation of $(2n)$ nuclei and the formation of conjugate nuclei $(n)+(n)$ is as prime a factor in the working of the diploidisation process in *Ustilago zeae* as it is in the Hymenomycetes.

The formation of conjugate nuclei in the diploid phase of the mycelium in the Smut Fungi has doubtless been inherited from basidiomycetous ancestors in which the conjugate nuclear arrangement was of high importance for the diploidisation process; and it may be that, in the Smut Fungi, the arrangement of the nuclei in the diploid mycelium in the form of conjugate pairs $(n)+(n)$ instead of as isolated nuclei $(2n)$, while still advantageous to some species, e.g. *Ustilago zeae*, in other species, e.g. *U. violacea* and *U. avenae*, has lost its primary significance.

The Exoascaceae. The Exoascaceae are a small group of highly specialised ascomycetous parasites. In *Taphrina epiphylla* and *T. Klebahnii*, each ascus contains eight haploid ascospores. Under favourable conditions, these ascospores bud and give off sprout-

¹ Hans Kniep, *Die Sexualität der niederen Pflanzen*, Jena, 1928, pp. 1-544.

² W. F. Hanna, "Studies in the Physiology and Cytology of *Ustilago zeae* and *Sorosporium reilianum*," *Phytopathology*, vol. xix, 1929, pp. 415-442.

conidia (yeast-like cells) which are able to multiply themselves by further budding. Each bud is haploid and has the same sexual constitution as the ascospore from which it was derived. When two sprout-conidia of opposite sex lie close together, one of them sends out toward the other a short process which fuses with the other conidium. Thereupon the nucleus of the first conidium passes into the second conidium. The second conidium then puts out a germ-tube which penetrates into the host and develops there into an extensive vegetative mycelium. This mycelium contains pairs of nuclei which apparently multiply by conjugate nuclear divisions and, finally, it gives rise to a hymenium composed entirely of asci situated directly on the surface of the host. Each young ascus contains a pair of conjugate nuclei which soon unite to form a fusion nucleus. This fusion nucleus then divides three times and so gives rise to eight haploid nuclei, one for each spore.¹

The life-history of a typical *Exoascus*, as outlined above, very much resembles that of *Ustilago violacea* and *U. avenae* already described. Just as in those Smut Fungi, so far as our present knowledge goes, there does not seem to be any possibility of the conjugate nuclei aiding the diploidisation process. If the formation of conjugate nuclei was of advantage for the diploidisation process to the non-parasitic ancestors of the *Exoascaceae*, that advantage may have ceased to exist when the *Exoascaceae* were definitely evolved.

The Pyrenomycetes and the Discomycetes. The Pyrenomycetes and the Discomycetes—the two largest groups of the Ascomycetes—have a vegetative mycelium which, so far as is at present known, differs from that of the Basidiomycetes in being *always haploid* and never diploid. Normally, the fruit-bodies of Ascomycetes are produced on a single haploid mycelium (monoecious species) or on two sexually interacting haploid mycelia (dioecious species) and, in a number of species, each fruit-body owes its origin to sexual organs—ascogonia and antheridia. In the vegetative mycelium of

¹ M. Wieben, "Infektion, Myzelüberwinterung und Kopulation bei Exoasceen," *Forschungen a.d. Geb. d. Pflanzenkrankheiten*, herausgeg. v. Schaffnit, Heft III, 1927, pp. 139–176 (cited from H. Kniep, "Vererbungsercheinungen bei Pilzen," *Bibliographia Genetica*, Bd. V, 1929, pp. 387–388).

the Pyrenomycetes and Discomycetes, therefore, the diploidisation of one haploid mycelium by one or a few nuclei derived from another mycelium of opposite sex, which is so characteristic of the Basidiomycetes, never takes place.

In certain Pyrenomycetes and in the Discomycetes, conjugate nuclei, while unknown in the vegetative mycelium, have been found to be present in the ascogenous hyphae which are pushed out from the ascogonium. In these hyphae cell-division accompanied by conjugate nuclear division and by the formation of hooks takes place and continues until the asci have been produced. In each young ascus the two nuclei of opposite sex fuse together. The fusion nucleus then undergoes three successive divisions thus originating eight haploid nuclei, one for each of the eight spores. Of the eight spores in an ascus, four are of one sex and the other four of opposite sex.¹

In some Pyrenomycetes, *e.g.* *Gnomonia leptostyla*, *Venturia inaequalis*, *Claviceps purpurea*, and in some Discomycetes, *e.g.* *Pyronema confluens*, *Ascobolus magnificus*, both kinds of sexual organs, ascogonia and antheridia, are developed; while in other Pyrenomycetes, *e.g.* *Polystigma rubrum*, *Gnomonia erythrostoma*, and in other Discomycetes, *e.g.* *Ascobolus furfuraceus*, *Ascophanus carneus*, *Cudonia lutea*, *Humaria granulata*, *Rhizina undulata* and *Thelebolus stercoreus*, ascogonia are developed but no antheridia.²

In the sexual process which goes on in Pyrenomycetes and Discomycetes, one or more nuclei derived from an antheridium or, in apandrous species, derived from some other cell form one or more pairs of conjugate nuclei $(n)+(n)$ in the ascogonium,³ and

¹ In some species, *e.g.* the Pyrenomycetes *Neurospora tetrasperma* investigated by B. O. Dodge (*Journ. Agric. Research*, vol. xxxv, 1927, pp. 289-305) and *Pleurage anserina* investigated by Miss E. Silver Dowding (*Annals of Botany*, vol. xlv, January, 1931), there are only four spores in each ascus, and each spore contains two nuclei of opposite sex and is bisexual. There are various other exceptions to the rule that in Pyrenomycetes and Discomycetes the ascus contains eight spores, but it is unnecessary to discuss them here.

² For the literature *vide* H. Kniep, *Die Sexualität der niederen Pflanzen*, Jena, 1928.

³ For the present, with Hans Kniep, I accept the conclusion of Claussen (as against that of R. A. Harper) that, in *Pyronema confluens*, the male and female nuclei, on meeting in the ascogonium, do not fuse but form conjugate pairs.

these pairs of nuclei move into the ascogenous hyphae where they undergo conjugate nuclear division which comes to an end when a sufficient number of ascus cells has been produced. We thus see that in the fruit-bodies of the Pyrenomycetes and Discomycetes, just as in the vegetative mycelia of the Basidiomycetes, the nuclei of opposite sex are attracted to one another to the extent of forming pairs which can divide conjugately but are inhibited from fusing with one another until the cells which are to form the spores have come into existence.

In a number of species of Pyrenomycetes and Discomycetes, the oogonium contains numerous nuclei and receives numerous nuclei from the antheridium ; and there is therefore a possibility that the conjugate nuclear arrangement in these fungi may be correlated, as it is in the Hymenomycetes, with the pairing of unequal numbers of nuclei of opposite sex. This possibility will now be discussed with special reference to *Pyronema confluens*.

The sexual organs of *Pyronema confluens* are of large size ; and, according to Claussen,¹ the ripe ascogonia and antheridia each contain some hundreds of nuclei. Hence we may assume that there is usually an inequality in the number of nuclei contained in an antheridium and its associated ascogonium. Claussen points out that the number of ascogonia which may unite with a single antheridium varies from one to three, and that this alone affords evidence that often there must be an inequality in the number of male and female nuclei which come together. Since, as we may assume, the number of male nuclei which enter an ascogonium is not equal to the number of female nuclei already present there, it might at first seem that, after the pairing of the male and female nuclei in the ascogonium has taken place, a certain number of nuclei, all male or all female, must remain unmated ; but, in view of what happens in the diploidisation process in the Hymenomycetes, it is not difficult to imagine how surplus male or female nuclei might be provided with mates. Let us assume, for example, that one hundred male nuclei derived from an antheridium that is united with the trichogynes of two or three ascogonia make their

¹ P. Claussen, "Zur Entwicklungsgeschichte der Ascomyceten. *Pyronema confluens*," *Zeitschrift f. Botanik*, Bd. IV, 1912, pp. 1-64.

way through one of the trichogynes into the subjacent ascogonium, and let us suppose that this ascogonium contains two hundred female nuclei. On coming into the ascogonium the one hundred male nuclei would be attracted to the female nuclei, but would be inhibited from fusing with them. Hence, if none of the nuclei underwent division, one hundred pairs of conjugate nuclei would be formed and one hundred female nuclei would be left without mates. However, since the one hundred male nuclei in the one hundred conjugate pairs of nuclei have not fused with their mates but have retained their identity, there is the possibility that any male nucleus of a conjugate pair that happened to be situated near an unpaired female nucleus might receive a stimulus from that nucleus, divide, and so provide an extra male nucleus that might pass to the unpaired female nucleus and so provide it with a mate. In this way it is conceivable that most or all of the one hundred female nuclei for which at first there were no male mates might come to have mates in the end. Similarly, if more male nuclei entered an ascogonium than there were female nuclei there, some of the female nuclei in the conjugate pairs might divide and so supply the unmated male nuclei with their appropriate mates.

Assuming that the diploidisation process takes place in the ascogonia of *Pyronema confluens* in the way that has been suggested, it is clear that an essential factor in its operation is that two nuclei of opposite sex, on coming together, do not fuse and form isolated $(2n)$ nuclei but remain associated as a pair of conjugate nuclei $(n)+(n)$, thus permitting every member of a pair of conjugate nuclei to retain its identity, so that it can divide independently of its fellow member whenever such a division will promote the diploidisation process.

The same inhibition that prevents two nuclei of opposite sex fusing in the ascogonium prevents them fusing in the ascogenous hyphae, where conjugate nuclear division takes place until the ascus cells are formed.

Enough has been said by way of suggestion as to how the organisation of the nuclei of Pyrenomycetes and Discomycetes as conjugate pairs $(n)+(n)$ instead of as isolated nuclei $(2n)$ may be of importance during the diploidisation process. It will be of

interest to see whether this suggestion will be accepted or rejected by workers on the Pyrenomycetes and Discomycetes in the course of their cytological investigations.

In concluding this chapter, the author desires to draw the reader's attention to a résumé of its contents given in the General Summary (pp. 302-305).

GENERAL SUMMARY

THE FOLLOWING IS A SUMMARY OF THE MORE IMPORTANT RESULTS OBTAINED DURING THE INVESTIGATIONS

PART I

Chapter I.—In the Curtus Sub-type : (1) the gills are parallel-sided and flanged at their edges ; (2) there are no cystidia on the gill-sides ; (3) the existence of the interlamellar spaces is secured not by cystidia but by the presence of gill-flanges, by an appropriate separation of the gills where they adjoin the pileus-flesh, and by the shallowness and sufficient rigidity of the gill-plates ; (4) the basidia are dimorphic ; (5) the fruit-bodies are very small and the top of the pileus becomes flattened before spore-discharge begins ; (6) the pileus-flesh and the gills become cleft from above downwards so as to form conspicuous radial sulcations on the top of the pileus ; and (7) autodigestion is slight and affects only the lower unsplit portion of each gill. The only representative of the Curtus Sub-type so far recognised is *Coprinus curtus*. *C. curtus* is identical with *C. plicatiloides* of Volume I.

The author gives a full account of the structure and mode of spore-discharge of *Coprinus curtus* accompanied by numerous illustrations, and he also provides a taxonomic description of the species.

Fruit-bodies of *Coprinus curtus* may be rendered sterile by fumes from fresh horse manure.

The pilear scales, which are minute, vary in colour from white to deep red. The pilocystidia and caulocystidia excrete drops of colloidal liquid.

The trama in the upper part of each long gill consists of outer ordinary cylindrical hyphae and of central oval or spherical cells. These central cells, as the pileus opens, swell up greatly and become the largest cells in the fruit-body. Hence they have been called *giant tramal cells*. Their function appears to be a mechanical one : by swelling up they doubtless help to split the upper part of each gill into two halves and so assist in the expansion of the pileus.

In horse-dung cultures in the laboratory, successive crops of fruit-

bodies expand their pilei and shed their spores about noon. This periodicity in fruit-body development is regulated by daylight.

The response of the stipe to heliotropic and geotropic stimuli in succession results in the pileus being pushed out from crevices between dung-balls into the open and then set in the best position for spore-discharge.

The spore-discharge period is very brief : it was found to range from about thirty minutes in very small fruit-bodies to about three and a half hours in very large fruit-bodies.

Chapter II.—In the *Plicatilis* Sub-type : (1) the gills are parallel-sided and do not have flanges at their edges ; (2) cystidia are present on the gill-sides but, in the unexpanded fruit-body, are not attached by both ends to opposing gills ; (3) the existence of the interlamellar spaces is secured in part by a suitable spacing of the gills where they adjoin the pileus-flesh and at their margins close to the stipe, and in part by the cystidia which act as guards and prevent adjacent gills from touching one another anywhere with their hymenial surfaces ; (4) before spore-discharge begins, the pileus expands umbrella-wise and adjacent gills become widely separated from one another, so that the cystidia project from the gill-sides like pegs ; (5) as the pileus opens, the pileus-flesh and gills become cleft from above downwards, so as to form conspicuous radial sulcations on the top of the pileus ; (6) the basidia are irregularly dimorphic-trimorphic ; (7) in the unexpanded pileus there is a short sheath of pileus-flesh surrounding the top of the pileus bounded at its margin by a collar made up of the inner ends of the gills ; and (8) autodigestion of the gills does *not* take place, so that the spore-freed portions of the gills are not destroyed from below upwards. The absence of autodigestion serves to distinguish the *Plicatilis* Sub-type from all other Sub-types. The only representative of the *Plicatilis* Sub-type so far recognised is *Coprinus plicatilis*.

The author gives a full account of the structure and mode of spore-discharge of *Coprinus plicatilis* accompanied by numerous illustrations, and he also provides a taxonomic description of the species.

Coprinus plicatilis failed to produce fruit-bodies when grown on sterilised horse-dung. The species appears to be graminicolous.

Coprinus hemerobius is regarded as merely a large form of *C. plicatilis*.

The hymenium, made up of dimorphic-trimorphic basidia, large sterile paraphyses, and cystidia, is typically coprinoid in structure.

The spores have three differing dimensions. In still air they fall at the rate of about 4.29 mm. per second. Their specific gravity is about 1.21.

In the open, successive crops of fruit-bodies expand their pilei daily about noon or in the early afternoon. This periodicity in fruit-body development is probably regulated by daylight.

The spores on the gills ripen and are discharged, as in other *Coprini*,

from below upwards; but the gills are not destroyed by autodigestion from below upwards. After spore-discharge is over, the gills are left intact and are whitish owing to the black spores having disappeared from the hymenium.

The four spores of five individual basidia were shot away from their sterigmata one after the other within fifteen seconds.

The non-autodigestion of the gills in *Coprinus plicatilis* is disadvantageous for the discharge of the spores only in a very limited degree, as in this species the upper halves of the gills split down their middle plane and the hymenium on the split halves looks more or less downwards, so that the spores on these halves can be discharged into the air without any mechanical hindrance.

The species most nearly related to *Coprinus plicatilis* appears to be *C. longipes* which comes up on old horse dung in laboratory cultures at Winnipeg. In *C. longipes* the gills undergo slight autodigestion at their edges.

Chapter III.—The relative efficiency of the Coprinus and Non-Coprinus Types of fruit-body organisation has been discussed. The Non-Coprinus Type, as represented by the Panaeolus Sub-type, produces about three times as many spores per unit of area of the hymenium as the Coprinus Type, but employs relatively much thicker gills on which to develop the hymenium. The Coprinus Type, as represented by large species such as *Coprinus comatus* and *C. atramentarius*, has much less pileus-flesh than the Non-Coprinus Type, as represented by *Psalliota campestris*.

The nature of Coprinus ink is discussed and the way of making it. The blackness of the ink is chiefly due to the presence of the spores, but is in part due to the liquid portion of the ink, which is brown. The brown colour of the spore-less juice is due to the action of oxidases.

The hyphal string in the centre part of the hollow stipe of *Coprinus comatus* has been illustrated. A record is made of the size of a giant fruit-body of *C. comatus* found by Dr. C. W. Dodge in Costa Rica.

An illustration is given of a pileus of *Coprinus niveus* which has excreted drops of liquid.

It has been shown that the Coprinus which grows on Beet and Mangel seeds is *Coprinus lagopus*, and a photograph of the fungus growing on Sugar-beet seeds is reproduced.

Chapter IV.—In *Coprinus sterquilinus*, passage of the spores down the alimentary canal of a horse is an essential feature in the life-history. Only those spores which are embedded in horse-dung balls succeed in producing mycelia which give rise to fruit-bodies. The results of experiments appear to indicate that, under natural conditions, wind-blown spores which settle on horse-dung after this has been deposited have practically no chance of ever producing mycelia which are effective in reproduction.

The presence of bacteria is not required for the germination of the spores of *Coprinus sterquilinus*.

The spores of *Coprinus sterquilinus* are able to germinate at a temperature below 20° C. From eighty to ninety per cent. of the spores germinated in a refrigerator in which the temperature varied from 5° to 10° C.

The spores of *Coprinus sterquilinus* are able to germinate, without having been dried or heated, immediately after they have been liberated from a fruit-body.

The struggle for possession of the substratum by mycelia derived from spores embedded in dung-balls is discussed.

In general, the spores of all Coprini germinate well in sterilised media of various kinds and, therefore, in the complete absence of bacteria.

Chapter V.—Attention is called to the mechanical problems involved in the fixation of hymenomycetous fruit-bodies to their substratum.

An analysis of the factors concerned in the mechanical fixation of the fruit-body of *Coprinus sterquilinus* in horse-dung masses has been attempted.

The stipe of *Coprinus sterquilinus* becomes fixed in its faecal substratum only owing to a combination of various external and internal factors for the fixation process. Light inhibits the development of all the tiny rudiments of fruit-bodies which happen to arise on the illuminated surface of the substratum, so that only those rudiments which happen to develop in dark recesses beneath or between dung-balls can possibly produce mature fruit-bodies. In the dark, a rudiment may continue its development if this is not inhibited by the growth of a more vigorous neighbouring rudiment. A favoured rudiment growing in the dark at a short distance below the surface of the substratum devotes its growth-energy to the development of a solid stout stipe-base, while the pileus which caps it remains small and conical, its development being delayed. A negative geotropic stimulus soon causes the stipe-base to push the rudimentary pileus upwards between the dung-balls or through the dung-mass into the light; and, as soon as the pileus has reached the light and with this the free upper surface of the substratum, the light inhibits the further growth in length of the stipe-base; and then the pileus and the hollow aerial stipe-shaft are developed to their full length in succession. The structure of the dung-mass, which consists of more or less spherical or oval lumps of considerable size piled together, is an obvious factor in fixation; for it provides the crevice or recess in which a favoured rudiment may begin its development and then, by mutual opposition of its parts, presses against the sides of the solid stipe-base so that the latter becomes firmly wedged in between the dung-balls, between or through which it has to grow. The very base or first-formed part of the stipe-base is attached to the mycelium within a dung-ball and to the mycelial stratum (with its mycelial strands)

which clothes the exterior of the dung-ball. The solid shaft of the stipe-base, which may be from 0.5 to 4.0 cm. long, according to the distance upwards through which the stipe-base grows to bring the rudimentary pileus into the light, is held laterally by the before-mentioned mutual pressure of opposing dung-balls, etc. Thus the whole solid stipe-base becomes fixed within the substratum in a most effective manner. Its hold upon the dung is doubtless rendered all the more complete by the densely packed hyphae which grow more or less radially outwards from all over the surface of the stipe-base and also from the lowest part of the stipe-shaft. These hyphae, which give a woolly or peronate appearance to the parts which they clothe, make their way into crevices between particles of the dung and to some extent contribute to the stability of the fruit-body as a whole. With the elucidation of the various factors which play a part in the fixation of a fruit-body, the problem of fixation for *Coprinus sterquilinus* may be considered to have been solved.

The upward pressure exerted by a growing fruit-body of *Coprinus sterquilinus* was measured and found to be nearly half a pound. The greater upward pressure of larger and more solid fruit-bodies of other agarics has been discussed.

In response to unilateral illumination, the stipe of *Coprinus sterquilinus* bends out of the vertical toward the light to the extent of only 8°–15°. A greater bending than this would be disadvantageous for the mechanical stability of the fruit-body as a whole. It is evident that, in deciding the exact position of the aerial stipe-shaft, the geotropic stimulus has a greater effect than the heliotropic.

PART II

Chapter I.—Social organisation exhibited by individuals of one and the same species has attained a high state of perfection in certain animals, *e.g.* the hive-bee, ants, termites, and man; but it is unknown in the Phanerogamia, the Pteridophyta, and the Bryophyta.

In some of the Thallophyta, namely, certain Algae, the Myxobacteriaceae, the Acrasieae, the Mycetozoa, and certain Fungi, the individuals of one and the same species become associated to form remarkable social communities.

In the Algae, the individuals of the same species may be associated in a common gelatinous investment as in *Aphanocapsa* and *Coelosphaerium*, or may build up a common gelatinous stalk as in *Dinobryon*, *Gomphonema*, and *Licmophora*, or may join to form a coenobium as in *Hydrodictyon* and certain *Volvocaceae*. The author is inclined to regard a *Volvox* not as a colony of individuals, but as a highly organised, multicellular, individual plant.

In the Myxobacteriaceae, *e.g.* *Chondromyces crocatus*, the individual bacteria by mass action build up an aerial gelatinous branched stalk or cystophore which ultimately bears cysts in which are packed living bacteria. Social organisation is exhibited by the bacteria in two ways: (1) in the common effort involved in the construction of a complex fructification, and (2) in that the bacteria which are enclosed in the stalk are sacrificed for the welfare of the bacteria enclosed in the cysts, which alone are able to reproduce the species.

In the Acrasieae, *e.g.* *Dictyostelium mucoroides*, the myxamoebae form an aggregate plasmodium in which the individuals retain their identity. Finally, a plasmodium builds up a long, sterile stalk crowned by a globular mass of naked spores. The myxamoebae which form the stalk never take a direct part in reproduction, but they assist reproduction by constructing an apparatus (the stalk) up which the spore-forming myxamoebae may ascend to take up a position which presumably is favourable for spore-dispersal.

In the Mycetozoa, social organisation is exhibited: (1) by the union of young plasmodia to form a larger compound plasmodium which acts as a whole in the formation of sporangia; (2) by zygotes, when beginning to feed, ingesting unpaired amoebulae and using them as food; and (3), as in *Arcyria pomiformis*, by the spores being differentiated into two kinds—the *spores proper* which reproduce the species, and the *spore-like cells* which are never disseminated but fill the stalk and mechanically strengthen the apparatus in which the true spores are developed and from which they are dispersed.

In most of the Phycomycetes, *e.g.* *Mucor*, *Pilobolus*, and *Saprolegnia*, apart from the requirements of sex, the individual mycelia of any species do not fuse together and do not display any social organisation.

In the Hymenomycetes—the leading group of the Higher Fungi—both in homothallic and in heterothallic species, any two mycelia, whatever their sexual phase, on coming into contact with one another fuse together hyphally, so that, in dung-balls, wood, leaves, and other substrata, many mycelia of one and the same species often combine to form a compound mycelium which is a three-dimensional network of hyphae. Such a compound mycelium acts as a unit in producing fruit-bodies.

Social organisation in the Hymenomycetes has been illustrated with special reference to *Coprinus sterquilinus*. A fruit-body of a *Coprinus*, as Brefeld has shown, owes its origin to a single cell of the mycelium. Where, therefore, as in *Coprinus sterquilinus*, say one hundred mycelia in a dung-ball have combined together to form a single compound mycelium which produces one large fruit-body, the fruit-body, genetically, is the product of one of the components of the compound mycelium but, nutritionally, is developed at the expense of all of the one hundred components: one of the components succeeds in reproducing itself,

while the other ninety-nine remain sterile and exhaust themselves in supplying the more fortunate mycelium with the necessary food-stuffs.

The biological advantage to a hymenomycetous species in forming compound mycelia which act as units in the production of fruit-bodies lies in these considerations: (1) that every mycelium, before it can produce a fruit-body, must attain a certain mass; (2) that, in a given substratum, it must often happen that many mycelia are present at one and the same time and that each of them by itself never attains a sufficiently large mass to produce a fruit-body; and (3) that, by means of hyphal fusions and a union of the mycelia, competition which would result in general sterility is replaced by co-operation which results in a fertility that is highly advantageous for the perpetuation of the species. The components of a compound mycelium which genetically remain sterile may be compared to the neuter bees in a hive which never reproduce themselves but are a prime factor in securing the persistence of the hive-bee species, *Apis mellifica*.

In the Pyrenomycetes, e.g. *Nectria Solani*, *Trichoderma lignorum* (a stage of *Hypocrea rufa*), and *Pleurage anserina*; in the Discomycetes, e.g. *Ascobolus magnificus*; and in the Fungi Imperfecti, e.g. *Colletotrichum trichellum*: hyphal fusions take place between adjacent mycelia derived from different spores, with the result that compound mycelia are formed. Doubtless such compound mycelia act as social units in the production of fruit-bodies, thus behaving in a manner comparable with that already described for the Hymenomycetes.

Hyphal fusions in the Hymenomycetes are of importance in the five following ways: (1) they convert every young mycelium into a network of such a kind that food materials can be conducted through it to fruit-bodies, sclerotia, etc., in diverse directions; (2) in heterothallic species, they place two mycelia of opposite sex in continuity, thus making possible the association of their nuclei in conjugate pairs; (3) in heterothallic species, they convert the haploid mycelia into a network of such a kind that, when a haploid mycelium is being diploidised by nuclei derived from another haploid mycelium, the nuclei can travel through the mycelium which is being diploidised by numerous and varied routes; (4) they convert all mycelia, whether haploid or diploid, into a network of such a kind that a mycelium, when injured by the breaking of some of its hyphae, still remains a unit and can act as such in the production of a fruit-body; and, finally, (5) in any single species, whether homothallic or heterothallic, they permit of any number of adjacent mycelia, whatever may be their sexual state, uniting to form a compound mycelium and thus acting as a social unit in the production of fruit-bodies and spores.

With a view to throwing further light on the relation between hyphal fusions and sexual phenomena in the Hymenomycetes, some additional remarks have been made.

Chapter II.—The term *diploidisation* has been introduced to designate the process by which a haploid cell is converted into a diploid cell, or a haploid mycelium into a diploid mycelium, by the formation of conjugate nuclei within the cell or within the mycelium. The diploidisation process is completed: in Hymenomycetes, in a haploid mycelium which is converted into a diploid mycelium; in Uredineae, in the spore-bed of the aecidium; in certain Smut Fungi, e.g. *Ustilago zaeae*, in a haploid mycelium within the host-plant; in Exoascaceae, in a sprout-conidium on the surface of the host-plant; and, in certain Pyrenomycetes and Discomycetes, in the ascogonium.

A haploid or diploid cell is said to *diploidise* a haploid cell when, through its agency, the latter becomes converted into a diploid cell containing conjugate nuclei $(n) + (n)$.

A haploid or diploid mycelium is said to *diploidise* a haploid mycelium when, through its agency, the latter becomes converted into a diploid mycelium containing conjugate nuclei $(n) + (n)$.

In experiments on the diploidisation process in *Coprinus lagopus*, when a tiny fragment of a haploid or diploid mycelium has been set at or just outside the periphery of a very large haploid mycelium with a view to converting the latter into a diploid mycelium, the large mycelium is said to have been *inoculated* with the tiny fragment of mycelium, and the tiny fragment of mycelium is spoken of as an *inoculum*.

The method of inoculating a large haploid mycelium several centimetres in diameter with a small haploid or diploid mycelium 1–2 mm. in diameter has been introduced as an aid to the study of the diploidisation process in *Coprinus lagopus* and other Hymenomycetes.

The *Coprinus lagopus* of the author's *Researches on Fungi* and of his pupils is identical with the *Coprinus fimetarius* of Mlle Bensaude, Kniep, Brunswik, Oort, and other workers on sex in the Hymenomycetes.

An individual fruit-body of *Coprinus lagopus* produces four sexually different kinds of spores, and these spores give rise to four kinds of haploid mycelia with the factorial composition (AB) , (ab) , (Ab) , and (aB) . By pairing (AB) with (ab) and (Ab) with (aB) , it is possible to obtain two kinds of diploid mycelia $(AB) + (ab)$ and $(Ab) + (aB)$ respectively.

Combinations of the type $(AB) \times (ab)$ and $(Ab) \times (aB)$ have been spoken of as *legitimate* combinations between two haploid mycelia; and combinations of the type $(AB) \times (AB) + (ab)$ and $(ab) \times (AB) + (ab)$ have been spoken of as *legitimate* combinations between a haploid mycelium and a diploid mycelium.

Combinations of the type $(AB) \times (Ab)$, $(AB) \times (aB)$, $(ab) \times (Ab)$, and $(ab) \times (aB)$ have been spoken of as *illegitimate* combinations between two haploid mycelia; and combinations of the type $(AB) \times (Ab) + (aB)$, $(ab) \times (Ab) + (aB)$, $(Ab) \times (AB) + (ab)$, and $(aB) \times (AB) + (ab)$ have been

spoken of as *illegitimate* combinations of a haploid mycelium with a diploid mycelium.

In what follows it has been assumed that a large haploid mycelium is becoming diploidised when clamp-connexions appear on, and a change from the wide-angled to the narrow-angled mode of branching is shown by, all its peripheral hyphae progressively proceeding away from, and on both sides of, the inoculum.

In legitimate combinations, a diploid inoculum can convert a large haploid mycelium into a diploid mycelium as smoothly, rapidly, and completely as a haploid inoculum.

In two legitimate combinations $(Ab) \times (Ab) + (aB)$ and $(AB) \times (AB) + (ab)$, the large haploid mycelia (Ab) and (AB) became diploidised by the diploid inoculum. A factorial analysis of the two diploidised haploid mycelia showed that they must have had the composition $(Ab) + (aB)$ and $(AB) + (ab)$ respectively.

In a legitimate combination between a large haploid mycelium and a small diploid inoculum, the large haploid mycelium can be diploidised by the diploid mycelium (1) when the inoculum is set at the *periphery* of the haploid mycelium, or (2) when the inoculum is set at the *centre* of the haploid mycelium.

In a legitimate combination, when a diploid mycelium is set between two haploid mycelia resembling those which combined to form the diploid mycelium, *e.g.* $(AB) + (ab)$ between (AB) and (ab) , the diploid mycelium diploidises the two haploid mycelia simultaneously.

In legitimate combinations, as the mycelia grow older under cultivation, the diploidisation of a large haploid mycelium by a haploid or diploid inoculum takes place less rapidly and sometimes incompletely.

In eight legitimate combinations of all possible kinds, a haploid or diploid inoculum converted a large haploid mycelium, 5.5–6.0 cm. in diameter when just inoculated, into a diploid mycelium in 3–4 days.

Since the development of a clamp-connexion on a haploid hypha is an indication that pairs of conjugate nuclei have just been established in the cells adjacent to the clamp-connexion, the rate of movement of nuclei derived from an inoculum through a large haploid mycelium can be calculated as soon as the time which has elapsed between the inoculation of the large haploid mycelium and the first appearance of clamp-connexions at the periphery of the large haploid mycelium at a measured distance from the inoculum has been determined.

In eight legitimate combinations of all possible kinds, the average speed with which nuclei derived from the inoculum moved through a large haploid mycelium (5.5–6.0 cm. in diameter when inoculated) from one side to the other was found to be : (1) with four *haploid* inocula (ab) , (AB) , (aB) and (Ab) , set on four large haploid mycelia (AB) , (ab) , (Ab) , and (aB) respectively, 0.93 mm. per hour ; and (2) with four *diploid* inocula $(AB) + (ab)$, $(AB) + (ab)$, $(Ab) + (aB)$, and $(Ab) + (aB)$, set on four

large haploid mycelia (*AB*), (*ab*), (*Ab*), and (*aB*) respectively, 0.89 mm. per hour.

When a large haploid mycelium (*AB*) was inoculated with a haploid mycelium of opposite sex (*ab*), the (*ab*) nuclei travelled through the haploid mycelium a distance of 6.0 cm. in 40 hours or at the rate of 1.5 mm. per hour. This was the maximum rate of movement observed in a combination of two haploid mycelia.

When a large haploid mycelium (*AB*) was inoculated with a diploid mycelium (*AB*)+(*ab*), the (*ab*) nuclei travelled through the haploid mycelium a distance of 7.7 cm. in 64 hours or at the rate of 1.2 mm. per hour. This was the maximum rate of movement observed in a combination of a haploid and a diploid mycelium.

A haploid mycelium of *Coprinus lagopus*, like the haploid mycelia of Hymenomycetes in general, is a three-dimensional network of hyphae. Hence, nuclei cannot travel from an inoculum through a large haploid mycelium in straight-line courses which are radial in respect to the inoculum, but must travel through the large haploid mycelium in very zig-zag courses. Hence, in the experiment already cited in which the nuclei travelled through a large haploid mycelium a distance of 6.0 cm. from the inoculum in 40 hours or at the rate of 1.5 mm. per hour, the speed of the nuclei in passing along the hyphae of the mycelial network probably attained at least 2.0 mm. per hour and may have been 2.0-3.0 mm. per hour.

The nuclei derived from a tiny mycelial inoculum, when advancing through a large haploid mycelium which they are diploidising, can travel through any part of the haploid mycelium, old or young, but they move more readily through a younger part than an older part.

At the periphery of a large haploid mycelium which had been inoculated with a haploid mycelium of opposite sex, the conversion of haploid into diploid hyphae, as indicated by the development of clamp-connexions and a change from the wide-angled to the narrow-angled mode of branching, was directly observed under the microscope as a result of continuous watching.

The number of nuclei of opposite sex required to diploidise all the hyphae which come to contain conjugate nuclei in a large haploid mycelium 6.0 cm. in diameter may well exceed 250,000.

On a diploid hypha fourteen successive clamp-connexions were formed in the course of twelve hours. Therefore a clamp-connexion was formed, and a conjugate nuclear division took place, every fifty minutes.

In *Coprinus lagopus* any mycelium can form hyphal fusions with any other mycelium.

In illegitimate combinations, as a rule, a large haploid mycelium can be converted into a diploid mycelium by a diploid inoculum but not by either of two haploid inocula derived from the two haploid mycelia which were mated to provide the diploid inoculum. Thus a large

haploid mycelium (Ab) was converted into a diploid mycelium by the diploid inoculum (AB) + (ab) but not by the haploid inoculum (AB) or by the haploid inoculum (ab). Why, in illegitimate combinations, a diploid mycelium is superior to either of its two haploid components in diploidising power remains to be determined by further investigation.

The diploidisation of a haploid mycelium of *Coprinus lagopus* by a diploid mycelium in a legitimate combination must often take place under natural conditions in dung-balls and is an advantageous phenomenon in that it increases the amount of diploid mycelium and the chances that diploid fruit-bodies instead of haploid will be produced.

The biological significance of conjugate nuclei in the Basidiomycetes and the Ascomycetes has been discussed.

The complete diploidisation of any multicellular and multinuclear hymenomycetous haploid mycelium through the agency of a single nucleus or relatively few nuclei derived from a haploid mycelium of opposite sex or from a diploid mycelium containing nuclei of opposite sex is absolutely dependent on the formation of conjugate nuclei.

The organisation of the nuclei in conjugate pairs (n) + (n) instead of as isolated nuclei ($2n$) in the diploid mycelium and fruit-body of the Hymenomycetes results, it is true, in delaying the fusion of nuclei of opposite sex until the basidia come into existence, but it has the great advantage that, in the diploid mycelium, each member of a pair of conjugate nuclei retains its identity, so that one member of a pair can divide independently of the other member of the pair whenever such a division is able to promote the diploidisation of a haploid mycelium.

In the light of what is actually known concerning the diploidisation process in the Hymenomycetes, suggestions have been put forth as to how diploidisation proceeds in the Uredineae, the Ustilaginaceae, the Tilletiaceae, the Exoascaceae, the Pyrenomycetes, and the Discomycetes.

EXPLANATION OF PLATES I-IV

PLATES I AND II

FIGS. 1-12 in Plates I and II represent an attempt made by the author, in the present state of our knowledge of the sexual processes of the Hymenomycetes, to visualise diagrammatically in *Coprinus lagopus* the mode of union of two haploid mycelia of opposite sex, (*AB*) and (*ab*), the production by these mycelia of diploidised cells, and the development of one of the diploidised cells into a diploid mycelium.

FIG. 1.—Two haploid mycelia of opposite sex, (*AB*) on right (black nuclei) and (*ab*) on the left (white nuclei), each derived from a single basidiospore (shown in the lower left part of the diagram), are growing in close proximity and are about to meet and fuse with one another. Note the simple septa (absence of clamp-connexions), the wide-angled mode of branching, the oidiophores with their groups of oidia, and the isolated nuclei.

FIG. 2.—The middle hypha of the (*ab*) mycelium has grown toward, and has come into contact with, the uppermost hypha of the (*AB*) mycelium. A fusion at the point of contact is about to take place.

FIG. 3.—Hyphal fusion between the two hyphae which were in contact has just taken place by dissolution of the two appressed cell-walls.

FIG. 4.—An (*ab*) nucleus has just moved toward the (*AB*) nucleus in the (*AB*) half of the compound cell. Thus a pair of conjugate nuclei (*AB*) and (*ab*) has been established, and the cell in which they lie may be said to have been diploidised.

FIG. 5.—The diploidised hypha has grown apically in length, and the pair of conjugate nuclei have advanced along it. They are about to divide conjugately, and the conjugate division will be accompanied by the formation of a clamp-connexion.

FIG. 6.—In the diploidised hypha a backwardly directed hook has been formed.

FIG. 7.—The (*AB*) nucleus has passed into the hook and is now alongside of the (*ab*) nucleus which is in the main hypha.

FIG. 8.—Conjugate division of the pair of conjugate nuclei (*AB*) and (*ab*) is now taking place, and the two daughter pairs of conjugate nuclei will soon separate from one another.

FIG. 9.—The two daughter pairs of conjugate nuclei have now separated from one another. The main hypha and the hook have each been divided by a septum. The two nuclei of one pair of the daughter conjugate nuclei are now in the terminal cell of the hypha, while the two nuclei of the other daughter pair are situated one as a temporary prisoner in the hook-cell and the other in the subterminal cell of the hypha.

FIG. 10.—The backwardly directed end of the hook has now fused with the subterminal hyphal cell, thus completing the formation of the clamp-connexion and allowing the (*AB*) nucleus which was imprisoned in the hook-cell to escape into the subterminal hyphal cell and there join its mate, the (*ab*) nucleus.

FIG. 11.—The diploidised hypha with the clamp-connexion and its branch have now considerably elongated, and the two pairs of conjugate nuclei have crept along them toward their centres. Both of these pairs are about to divide conjugately.

FIG. 12.—The two cells containing conjugate nuclei shown in Fig. 11 have now developed into a diploid mycelium consisting of seven cells which are increasing in number. In the two upper right hyphae of the diploid mycelium the development of clamp-connexions and conjugate nuclear divisions are in progress. In the diploid mycelium, as contrasted with the haploid mycelium, note the septa accompanied by clamp-connexions, the narrow-angled mode of branching, the entire absence of oidiophores and oidia, and the presence in every cell of a pair of conjugate nuclei. The diploid mycelium after growing further, might give rise to a diploid fruit-body like that shown above. The spores which the fruit-body is represented as discharging would consist of all of the four possible sexual kinds: (*AB*), (*ab*), (*Ab*), and (*aB*).

For the sake of simplicity, in Figs. 1–12 only one diploidised cell and its development into a diploid mycelium has been represented; but, in reality, two haploid mycelia of opposite sex mutually diploidise one another so that a pair of conjugate nuclei comes to be present in the end-cell of every growing hypha. Thus every end-cell, after being diploidised, could have been represented as developing in a manner similar to that shown for the single diploidised cell of Fig. 4.

PLATES III AND IV

FIGS. 1–12 in Plates III and IV represent an attempt made by the author, in the light of our present knowledge of the sexual processes of the Hymenomycetes, to visualise diagrammatically in *Coprinus lagopus* the mode of union of a diploid mycelium (*AB*)+(*ab*) with a haploid mycelium (*ab*), and some of the stages involved in the diploidisation of the haploid mycelium (*ab*) by the diploid mycelium (*AB*)+(*ab*).

FIG. 1.—Two mycelia are represented: on the left is a diploid mycelium (*AB*)+(*ab*), and on the right a haploid mycelium (*ab*). The two mycelia are about to come into contact with one another and to fuse with one another. In the diploid mycelium (left) note the septa accompanied by clamp-connexions, the narrow-angled mode of branching, the entire absence of oidiophores and oidia none of which will be produced on it, and the presence in every cell of a pair of conjugate nuclei. In the haploid mycelium (right) note the plain septa (without clamp-connexions), the wide-angled mode of branching, and the isolated nuclei. In the haploid mycelium oidiophores and oidia are absent only because the hyphae are too young to produce them.

The central hypha of the diploid mycelium (*AB*)+(*ab*) is growing toward, and is about to come into contact with, a non-terminal cell of one of the hyphae of the haploid mycelium (*ab*).

FIG. 2.—The central hypha of the diploid mycelium has now come into contact with a hypha of the haploid mycelium. A fusion at the point of contact is about to take place.

FIG. 3.—A hyphal fusion between the two hyphae which were in contact has just taken place by dissolution of the two appressed cell-walls, and already the (*AB*) nucleus of the diploid half of the compound cell is dividing and forming two daughter nuclei.

FIG. 4.—The division of the (*AB*) nucleus has been completed, and one of the daughter (*AB*) nuclei has moved into the (*ab*) half of the compound cell and has become associated with the (*ab*) nucleus there present. Thus a pair of conjugate nuclei has been established in one of the cells of the haploid mycelium (*ab*), which cell may be said to have become diploidised.

FIG. 5.—The (*AB*) nucleus of the newly established conjugate pair is now dividing and one of the daughter (*AB*) nuclei is destined to pass into a neighbouring (*ab*) cell.

FIG. 6.—One of the daughter (*AB*) nuclei of Fig. 5 has now passed into a neighbouring (*ab*) cell and has thus diploidised it. The passage from one cell to the other was made possible by the breaking down of the septum between them, but such a breaking-down of the septum has not been represented in the diagram. A septum cutting off the diploid mycelium (*AB*)+(*ab*) from the original haploid mycelium (*ab*) is now shown.

FIG. 7.—The two (*AB*) nuclei in the recently diploidised cells of the haploid mycelium are now dividing.

FIG. 8.—Two of the daughter (*AB*) nuclei shown in process of formation in Fig. 7 have now moved into neighbouring cells and have thus diploidised them. One of these cells (on the right) is a terminal one, and already the pair of conjugate nuclei there established is about to divide, for a hook for the reception of one of the nuclei has been formed.

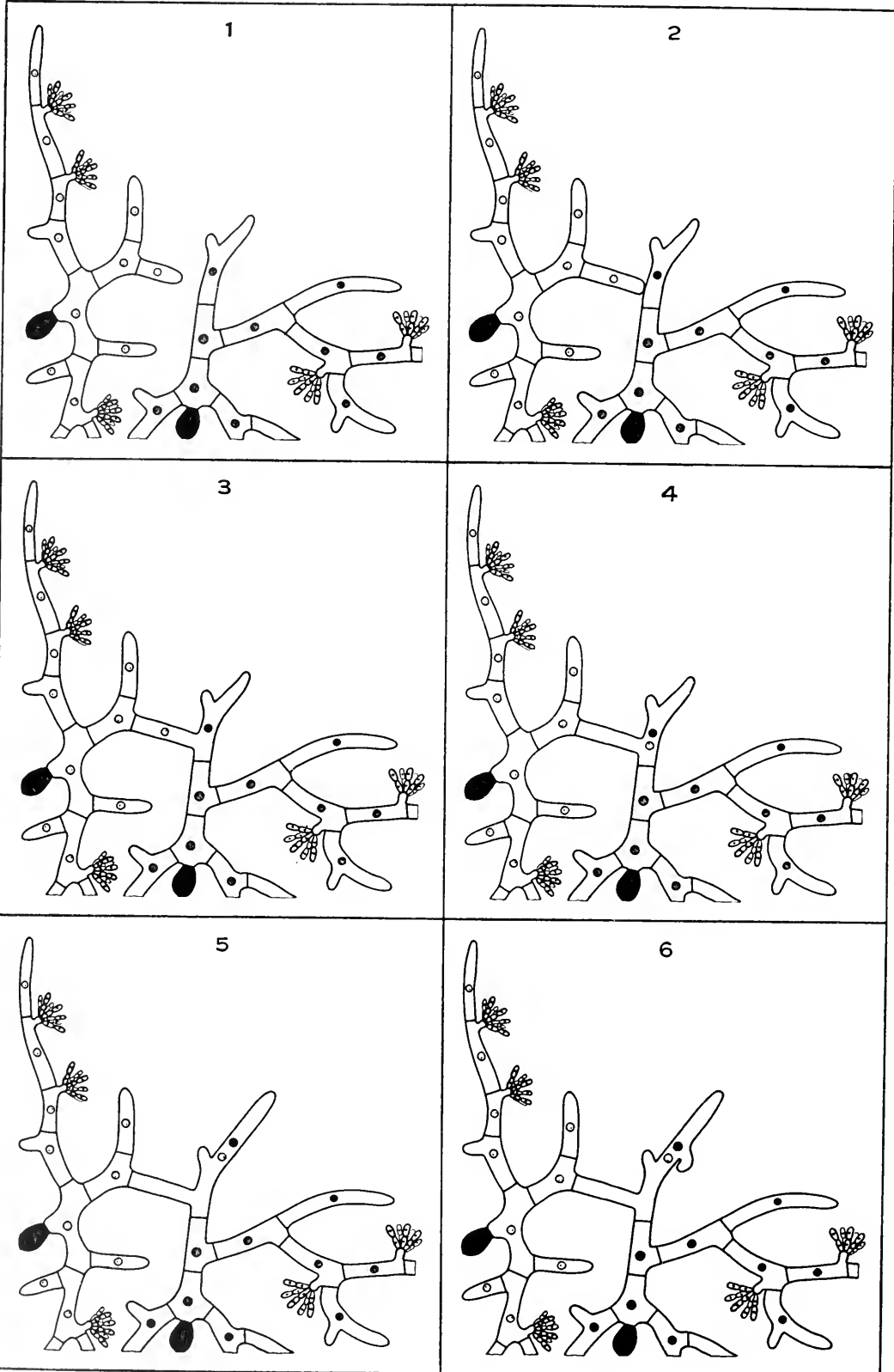
FIG. 9.—In the main hypha of the originally haploid mycelium (*ab*) two more divisions of (*AB*) nuclei are now in progress; while, in the end cell of the hypha on the right, conjugate division of the pair of conjugate nuclei is well advanced.

FIG. 10.—The main hypha of the originally haploid mycelium (*ab*) and its two branches have become completely diploidised, and conjugate division of the pairs of nuclei in its end cells, accompanied by the formation of a clamp-connexion, is taking place or is about to take place. A separate (*ab*) hypha on the right is still in the haploid condition, but a branch of it is growing toward the central already-diploidised hypha and is about to fuse with it. When this hyphal fusion has been established, the now separate haploid hypha (*ab*) will soon become diploidised.

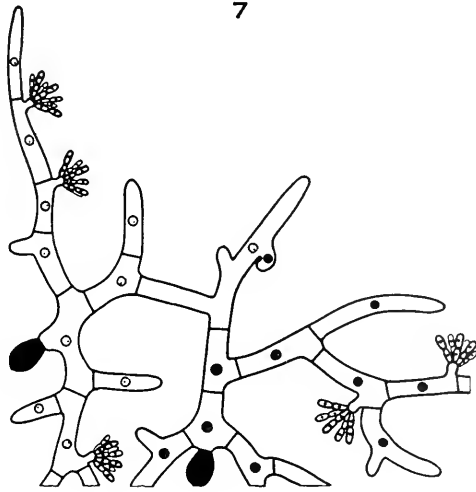
FIG. 11.—The central diploidised hypha and one of its branches have each formed an additional clamp-connexion. The union of a branch of the still haploid hypha (*ab*) on the right with the central diploidised hypha has now taken place. An (*AB*) nucleus is dividing in the connecting hypha. One of the daughter (*AB*) nuclei will pass into the (*ab*) hypha and there begin the diploidisation of its cells.

FIG. 12.—The whole of the originally haploid mycelium (*ab*) has now become diploidised except a short hypha (below) of which three cells have been shown; but a very short branch of the diploidised central hypha is seen growing toward it in the extreme lower right-hand corner of the diagram. This very short branch will meet and fuse with the short (*ab*) hypha; and, after the fusion has taken place, the cells of the short (*ab*) hypha will soon become diploidised.

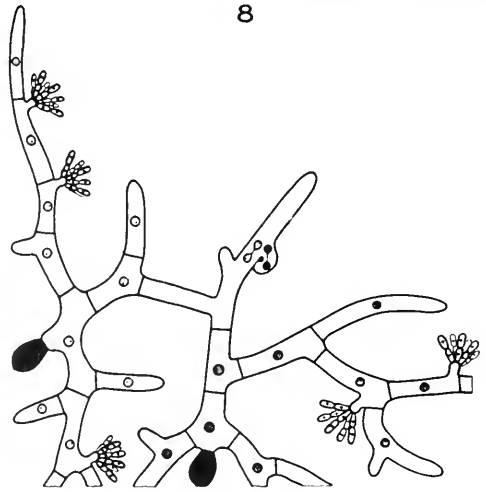
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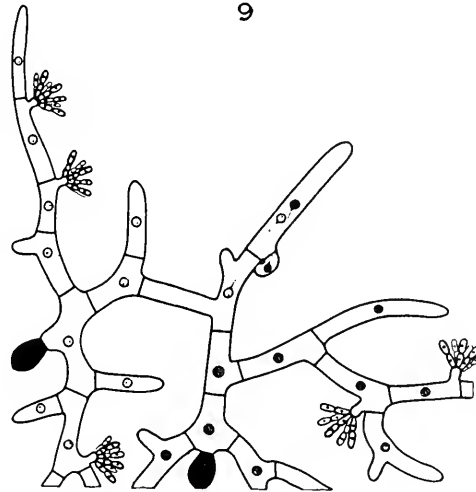
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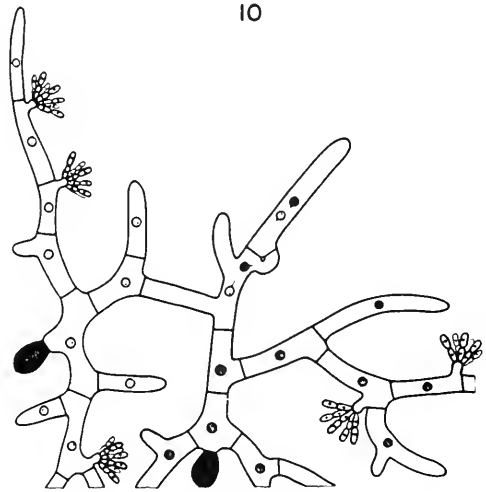
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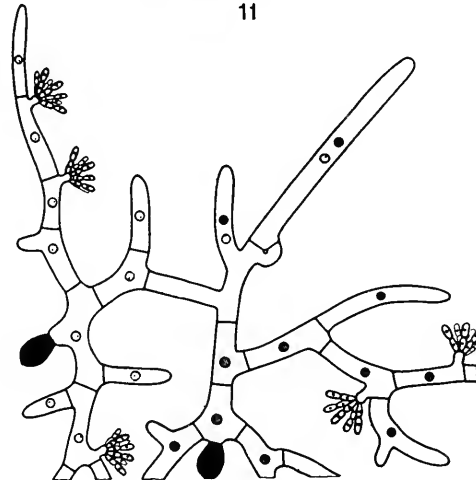
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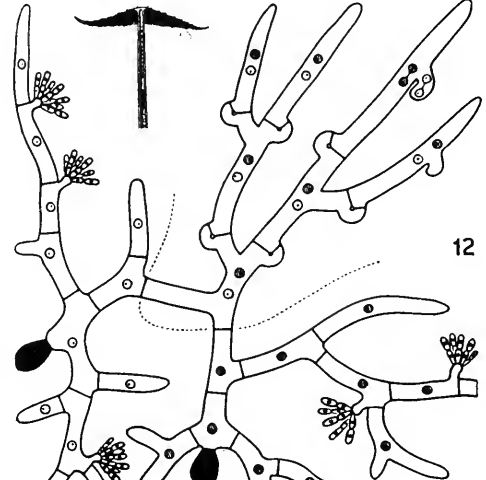
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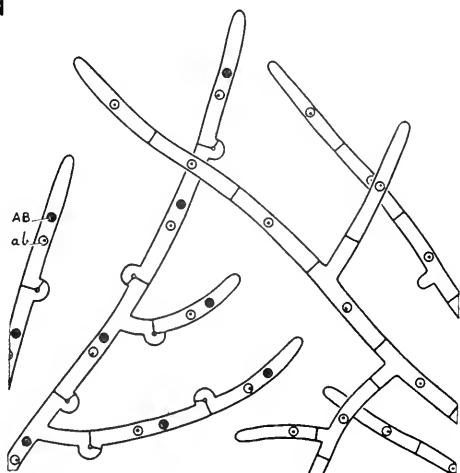
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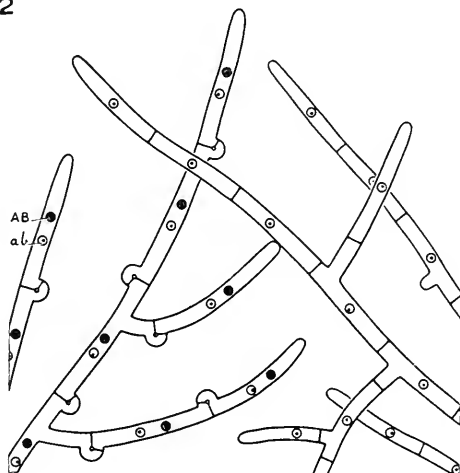
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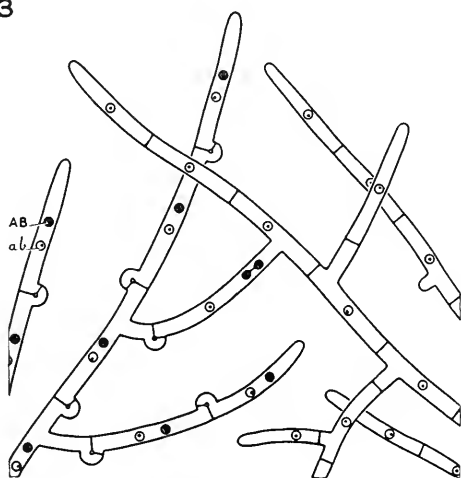
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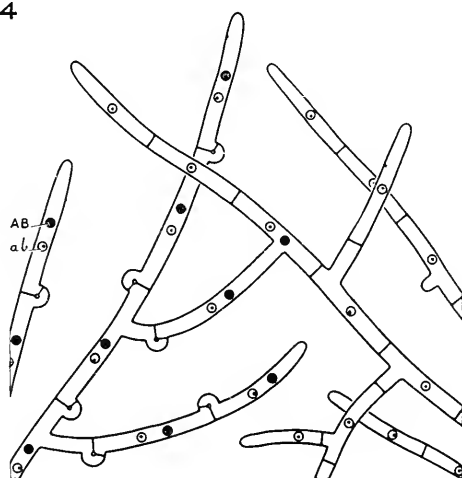
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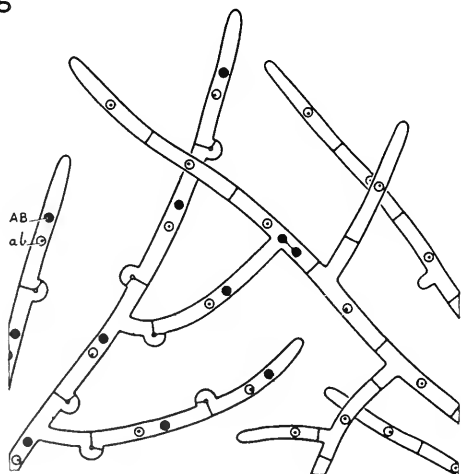
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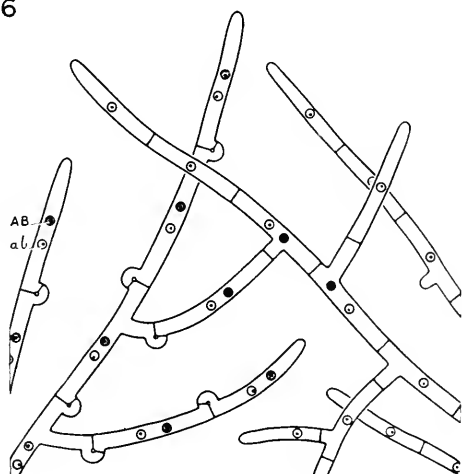
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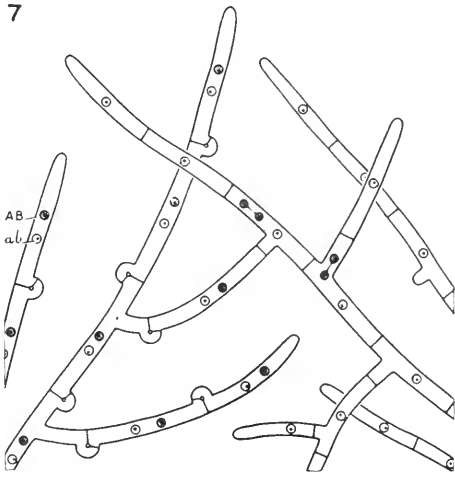
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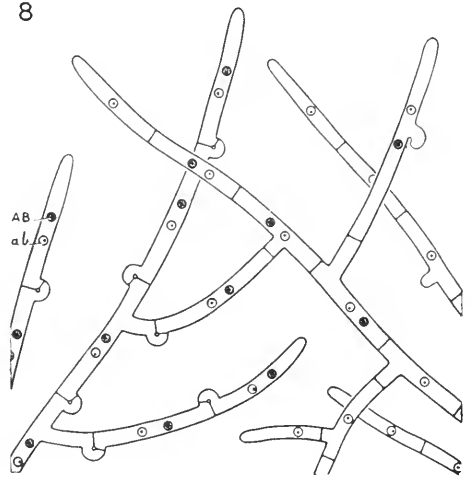
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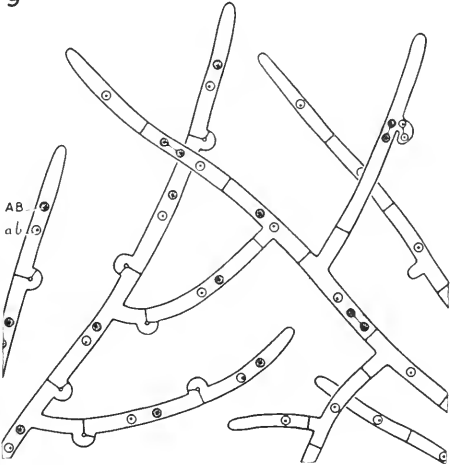
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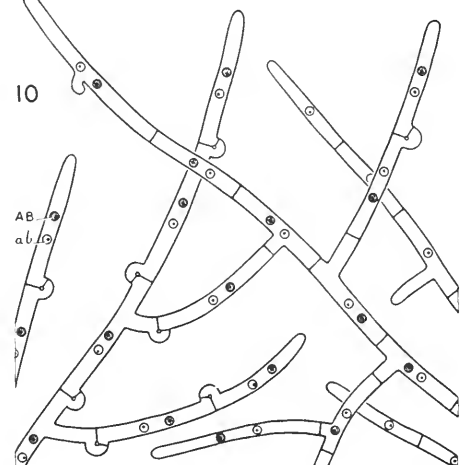
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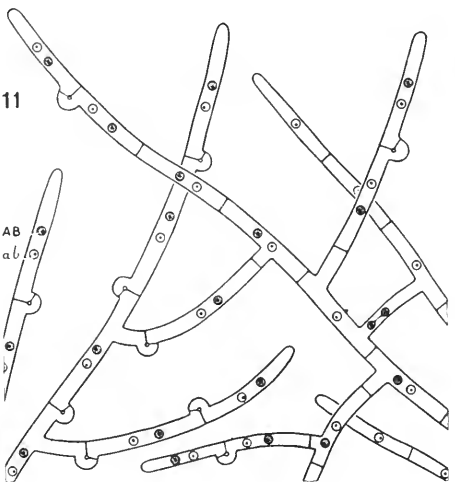
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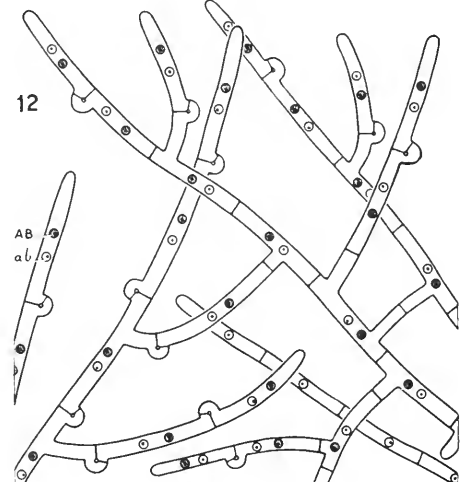
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11



12



GENERAL INDEX

- ABNORMALITIES, basidia, 44, 46
 „ drop-excretion, 31-32, 54
 „ sterile fruit-bodies, 9
 Abortion of fruit-body rudiments, 102-104, 160
 Acrasieae, social organisation, 144-146, 299-300
 Aequi-hymeniiferae, and evolution, 32
 Aequi-hymeniiferous Type, 63
 Agaricineae, coprophilous, 85
 „ fixation of fruit-bodies, 93-94
 „ heliotropism, 125, 126
 „ nuclei in fruit-bodies, 266
 Age and diploidising power of haploid mycelia, 212-213, 303
 Aggregate plasmodium, 146
Aleurodiscus polygonius, kinds of spores, 270
 Algae, social organisation, 139, 140-143, 299
 „ union of nuclei, 188
 Allen, Ruth F., on *Puccinia graminis*, 286
Amanita crenulata, heliotropism, 125
Amanita phalloides, heliotropism, 125
Amanita rubescens, fruit-body fixation, 93-94
Anellaria separata, fruit-body fixation, 136
 Anheliotropism, in young fruit-bodies, 123
 Animals, gametes and their union, 269
 „ nuclei of zygote, 269
 „ sexual process in, 274
 Antheridia, absence in certain Ascomycetes, 290
 Apandrous Ascomycetes, 290
Aphanocapsa Grevillei, illustration, 142
Apis mellifica, 168, 169, 301
 Arcyria, spore-like cells, 149
Arcyria pomiformis, illustration, 151
 „ „ social organisation, 149-152, 300
Armillaria mucida, kinds of spores, 270
Armillaria Sub-type, 63
Ascobolus furfuraceus, ascogonia, 290
Ascobolus magnificus, illustrations, 179, 180
 „ „ sexual organs, 290
 „ „ social organisation, 177-178, 180
Ascobolus pulcherrimus, hyphal fusions, 172
 Ascogenous hyphae, 188
 Ascogonia, in Ascomycetes, 290
 Ascomycetes, and conjugate nuclei, 188
 „ haploid mycelia of, 289
Ascophanus carneus, ascogonia, 290
 Ascus, and sex of spores, 290
 Atkinson, G. F., on *Coprinus curtus*, 11
 „ „ photographs by, 7-12
 Autodigestion, absence in *Coprinus plicatilis*, 35, 55-58, 297
 „ and raising of pileus, 73
 „ in *Coprinus curtus*, 28-29
 „ in *Coprinus longipes*, 60
 „ significance of, 55, 56
 BACILLARIALES, social organisation, 143
 Bacteria, and germination of spores of Coprini, 85-89, 90, 91, 298
 „ of Myxobacteriaceae, 143-144, 147
 Baden, M. L., on germination of spores of *Coprinus sterquilinus*, 85, 89-90
 Basidia, abnormal, 44
 „ and conjugate nuclei, 188
 „ containing one haploid nucleus, 266
 „ three sexual kinds in *Coprinus lagopus*, 273-274

- Basidia, trimorphic, 38, 44, 50
 „ two sexual kinds in *Coprinus Rostrupianus*, 274
- Basidiomycetes, and conjugate nuclei, 187-188, 269
- Basidiospore, a haploid cell, 281
- Basidiospores, mode of discharge and phylogeny, 281
 „ produce haploid mycelia, 188
- Basidium, diagram of, showing nuclei, 272
 „ fusion of nuclei and reduction in, 272
 „ successive discharge of spores from, 52-53
- Bees, social organisation, 149, 167-169, 301
- Beet seeds and *Coprinus lagopus*, 74-77
- Bennett and Murray, illustration from, 144
- Bensaude, M., and *Coprinus lagopus*, 191-193, 302
 „ „ dedication of book to, *vide* Preface
 „ „ illustration by, 155
 „ „ on clamp-connexions, 155, 243
 „ „ on *Coprinus fimetarius*, 191-193, 302
- Biological significance, of a diploid mycelium diploidising a haploid, 188-189, 263-268, 305
 „ „ of conjugate nuclei, 188-189, 268-293, 305
- Bisby, G. R., and *Coprinus plicatilis*, 36
 „ „ and the fungi of Manitoba, 58
- Blackman, V. H., and H. C. I. Fraser, on Uredineae, 282
 „ „ on the sexual process in the Uredineae, 282
- Bolbitius Sub-type, 63
- Botanical Congress, International, at Cambridge, 190
- Boudier, E., and *Coprinus ink*, 71-72
- Brefeld, O., and *Coprinus lagopus*, 181
 „ „ on *Coprinus stercorarius*, 169
 „ „ on *Dictyostelium*, 146
 „ „ on *Hypocrea rufa*, 173
- Brefeld, O., on origin of *Coprinus* fruit-bodies, 101, 164, 166, 167, 168
 „ „ on *Peziza Sclerotiorum*, 172-173
- Bridging hypha, 154
- Brodie, H. J., and *Coprinus lagopus*, 190, 204
 „ „ illustrations of oidia by, 197, 198, 200
 „ „ on oidia and insects, 286
- Brunswick, H., and *Coprinus fimetarius*, 193
 „ „ on *Coprinus curtus*, 6-7
 „ „ on durchbrechungskopulationen, 250
- Bryophyta, no social organisation in, 139
 „ nuclei in diploid phase, 269
 „ union of nuclei, 188
- Bulliard, P., on *Coprinus ink*, 70-71
- CALCIUM oxalate crystals, 15
- Carpenter, Dr., illustration by, 143
 „ „ on upward pressure exerted by fungi, 120
- Carter, N., illustrations copied by, 155, 166, 167, 168
- Caulocystidia, 17
- Cheilocystidia, 46
- Chemotropism, and hyphal fusions, 154
- Chimaerae, haploid, 250
- Chlorophyceae, social organisation, 140
- Chondromyces crocatus*, illustrations, 147, 148
 „ „ social organisation, 143-144
- Christman, A. H., on sexuality of Rust fungi, 283
- Chromosomes, reduction, 188, 272
- Chrysophyceae, social organisation, 140
- Churchman, S. G., and spore germination, 85-89
- Clamp-connexions, and conjugate nuclear divisions, 271
 „ and diploidy, 157
 „ and the diploidisation process, 240
 „ as a criterion of sex, 198-203
 „ illustrations, 155, 202, 271, 278, 279, 280, Plates I-IV
 „ in Hymenomycetes, 152-155
 „ in Smut Fungi, 287

- Clamp-connexions, outward and visible sign of presence of conjugate nuclei, 157, 271
 ,, time taken for formation of, 243
- Claussen, P., on *Pyronema confluens*, 290, 291-292
- Claviceps purpurea*, sexual organs of, 290
- Coelosphaerium Kützingianum*, illustration, 142
- Cohn, illustration by, 144
- Collar, formed by gills, 42, 62
- Colletotrichum trichellum*, illustrations, 180, 181
 ,, ,, social organization, 179-181
- Colley, H. R., on sexuality of Rust fungi, 283
- Colloidal drops, excretion of, 16-18
- Collybia longipes*, fruit-body fixation, 94
- Collybia platyphylla*, fruit-body efficiency, 65-66
 ,, ,, illustration, 66
- Collybia radicata*, fruit-body fixation, 94
 ,, ,, pseudorhiza, 113
- Collybia velutipes*, heterothallic, 171
- Compatibility, sexual, 204
- Compatible haploid and diploid mycelia, 245-246, 303
- Competition and co-operation between mycelia, 167
- Compound mycelia, and sex, 171-172
 ,, ,, nature of, 163
- Conjugate nuclear division, and clamp-connexions, 271
 ,, ,, ,, diagrams of, 155, 271, 278, Plates I-IV
 ,, ,, ,, frequency of, 243, 304
 ,, ,, ,, in *Exoascaceae*, 289
 ,, ,, ,, in *Pyrenomycetes* and *Discomycetes*, 290-293
 ,, ,, ,, in Rust fungi, 281
 ,, ,, ,, in Smut fungi, 287
- Conjugate nuclear division, nature of, 187-188
 ,, ,, ,, significance, 188-190
 ,, ,, ,, time required for, 243
- Conjugate nuclei, biological significance of, 188-189, 268-293, 305
 ,, ,, cytological observations on, 216
 ,, ,, diagrams showing, 155, 271, 272, 277, 278, 279, 280, Plates I-IV
 ,, ,, increased by conjugate nuclear division, 270-271
 ,, ,, introductory remarks on, 187-189
 ,, ,, their identity preserved, 189
- Conversion of haploid into diploid hyphae, 237-241, 275-278, 304
- Cooke, M. C., on upward pressure exerted by fungi, 120
- Coprini, and herbivorous animals, 85
 ,, homothallic, do not produce oidia, 286
- Coprini and Non-Coprini, growing together, 68
- Coprinus, and bacteria, 91
 ,, ink of, 69-72
- Coprinus and Non-Coprinus Types compared, 63-69, 297
- Coprinus atramentarius*, and *C. curtus*, 21, 26, 29
 ,, ,, and *C. plicatilis*, 45, 54, 56
 ,, ,, flesh of, 4
 ,, ,, fruit-body efficiency, 65-67
 ,, ,, fruit-body size, 32
 ,, ,, illustrations, 66, 71
 ,, ,, ink of, 69-72
- Coprinus comatus*, and bacteria, 91
 ,, ,, and *C. curtus*, 19, 21, 26, 29, 32
 ,, ,, and *C. plicatilis*, 50, 56
 ,, ,, and *Psalliota campestris*, 66-68

- Coprinus comatus*, and *Stropharia semi-globata*, 63-65
- " " fruit-body efficiency, 66-68
- " " giant fruit-body, 74
- " " heliotropism, 124-125
- " " hymenium and spores, 64
- " " hyphal string in stipe, 72-73
- " " illustrations, 64, 73
- " " ink of, 69-72
- " " pileus-flesh, 4, 73
- Coprinus curtus*, and bacteria, 91
- " " and *C. atramentarius*, 21, 26, 29
- " " and *C. comatus*, 19, 21, 26, 29
- " " and *C. ephemerus*, 12, 21
- " " and *C. lagopus*, 21
- " " and *C. micaceus*, 18, 21
- " " and *C. narcoticus*, 15
- " " and *C. niveus*, 21
- " " and *C. plicatilis*, 14, 18, 21, 40, 44, 49, 50, 51, 57-58
- " " and *C. Rostrupianus*, 32, 54
- " " and *C. stercorearius*, 15
- " " and *C. sterquilinus*, 19, 21, 28, 29, 32
- " " and fumes of fresh manure, 9
- " " and *Psathyrella disseminata*, 16
- " " caulocystidia, 17-18
- " " cultures of, 4-9
- " " developmental periodicity, 23-25
- " " discharge of spores, 28-32
- " " dwarf fruit-bodies, 18
- " " excretion from hairs, 16-18
- " " fruit-body size, 32
- " " giant tramal cells, 20-21, 22, 30, 295
- " " gills, 18-19
- " " heliotropism, 125, 126
- " " how obtained, 4
- " " hymenium, 19-20
- " " illustrations, twenty, 3-31
- " " no clamp-connexions in, 8
- Coprinus curtus*, opening of pileus, 26-28
- " " pilear flesh, 18
- " " pilear scales, 13, 14-15
- " " pilocystidia, 15-17
- " " ripening of spores, 26
- " " sexuality of, 6-8
- " " spores, 21, 23
- " " stipe, 25-26
- " " summary, 295-296
- " " taxonomic description, 11-14
- Coprinus domesticus*, sexuality and hyphal fusions of, 184
- Coprinus ephemerus*, and bacteria, 91
- " " and *C. curtus*, 12
- " " fruit-body size, 32
- " " on unsterilised horse dung, 84
- Coprinus fimetarius*, and *C. lagopus*, 191-193, 302
- " " durchbrechungskopulationen, 250
- Coprinus hemerobius*, and *C. plicatilis*, 38-40
- " " illustration, 43
- " " spores of, 47
- Coprinus ink*, 69-72
- Coprinus lagopus*, account of sexual process in, 187-281
- " " and bacteria, 91
- " " and *C. curtus*, 21
- " " and *C. plicatilis*, 45, 54
- " " and *C. sterquilinus*, 82, 84
- " " basidium and its nuclei, 272-274
- " " biological significance of conjugate nuclei, 268-281, 305
- " " biological significance of diploidisation of a haploid by a diploid, 188-189, 263-268, 305
- " " by whom used for investigations on sex, 190-192, 302
- " " conversion of haploid into diploid hyphae, 237-241, 275-278, 304
- " " diploidisation of a haploid mycelium by a diploid, 187-268, 304, 305

- Coprinus lagopus*, dwarf fruit-bodies, 14, 23
- " " frequency of conjugate nuclear division, 243
- " " haploid fruit-bodies, 235, 265-266
- " " heterothallic, 171
- " " hyphal fusions, 154, 169-171, 184, 243-246
- " " illegitimate combinations, 247-263
- " " illustrations, 76, 170; forty-two, 191-280, Plates I-IV
- " " incompatible combinations, 245-263
- " " kinds of mycelia, 203-204
- " " kinds of spores, 194, 270
- " " modes of pairing mycelia, 204-213
- " " number of radial hyphae diploidised, 241-242
- " " on Beet seeds, 74-77
- " " origin of fruit-body, 101
- " " progressive diploidisation, 275-278, 307-309
- " " rate of growth of mycelium, 199
- " " rate of movement of nuclei, 213-229, 303-304
- " " segregation of sex factors, 272-274
- " " septa, destruction of, 228, 275, 277, 280
- " " sex-factor analyses, 234-237, 250-252
- " " sexual process in, 187-281
- " " summary, 302-305
- " " synonymy, 190-193, 302
- Coprinus longipes*, and *C. plicatilis*, 58-60, 62, 297
- " " illustrations, five, 57-59
- Coprinus macrorrhizus*, and bacteria, 91
- " " pseudorhiza, 113
- Coprinus micaceus*, and *C. curtus*, 18, 21
- " " and *C. plicatilis*, 40, 50, 51
- " " pilocystidia, 43
- Coprinus narcoticus*, and calcium oxalate, 15
- " " homothallism and oidia, 286
- Coprinus niveus*, abnormal basidia, 44
- " " and bacteria, 91
- " " and *C. curtus*, 21
- " " and *C. plicatilis*, 46, 50, 56
- " " basidial trimorphism, 44
- " " excretion of water, 74, 75
- " " heliotropism, 125, 126
- " " illustration, 75
- Coprinus nycthemerus*, 76
- Coprinus picaceus*, and *C. plicatilis*, 56
- " " durchbrechungskopulationen, 250
- " " fixation of fruit-body, 93-94
- " " fruit-body size, 32
- " " ink of, 69
- Coprinus pilosus*, 75
- Coprinus plicatilis*, abnormal basidia, 44
- " " absence of autodigestion, 44, 51, 55-58, 297
- " " and autodigestion of cystidia, 54-55
- " " and bacteria, 91
- " " and *C. atramentarius*, 45, 50, 54, 56
- " " and *C. comatus*, 50, 56
- " " and *C. curtus*, 14, 18, 21, 40, 44, 49, 50, 51, 57-58
- " " and *C. hemerobius*, 38-40
- " " and *C. lagopus*, 45, 50, 54
- " " and *C. longipes*, 58-60, 62, 297
- " " and *C. micaceus*, 40, 50
- " " and *C. niveus*, 46, 50, 56
- " " and *C. picaceus*, 56
- " " and *C. stercorarius*, 56

- Coprinus plicatilis*, and *C. sterquilinus*, 50, 53, 54
- „ „ and *Marasmius oreades*, 55
- „ „ and *Panaeolus campanulatus*, 54
- „ „ and *Psalliota campestris*, 55
- „ „ cultures, 37-38
- „ „ cystidia, 45-46
- „ „ developmental periodicity, 49
- „ „ drop-excretion, 53
- „ „ expansion of pileus, 41-42
- „ „ generic position, 55
- „ „ geographical distribution, 36
- „ „ gill-collar, 42, 62
- „ „ gills, 44-46
- „ „ hymenium, 44-47
- „ „ illustrations, thirteen, 35-51
- „ „ in the field, 36, 51-52
- „ „ pileus, 40-43
- „ „ pileus never revolute, 40, 51, 58
- „ „ pilocystidia, 43
- „ „ rate of fall of spores, 47-48
- „ „ relation with substratum, 36-37
- „ „ ripening and discharge of spores, 49-55
- „ „ spore - discharge period, 52
- „ „ spores, 46-48
- „ „ stipe, 48-49
- „ „ summary, 296-297
- „ „ taxonomic description, 60-62
- „ „ trimorphic basidia, 44-45
- Coprinus plicatiloides*, 11, 125
- Coprinus radians*, sexuality, 184
- Coprinus Rostrupianus*, and bacteria, 91
- „ „ and *C. curtus*, 32, 54
- „ „ kinds of basidia, 274
- „ „ kinds of spores, 270
- Coprinus Rostrupianus*, sexuality and hyphal fusions, 184
- Coprinus stercorarius*, and bacteria, 91
- „ „ and calcium oxalate, 15
- „ „ and *C. plicatilis*, 56
- „ „ change to diploid phase, 186
- „ „ homothallism and oidia, 286
- „ „ hyphal fusions, 169
- „ „ illustrations of mycelium, 166-168
- „ „ origin of fruit-body, 101, 164, 166-168
- Coprinus sterquilinus*, and *C. curtus*, 19, 21, 28, 29, 32
- „ „ and *C. lagopus*, 82, 84
- „ „ and *C. plicatilis*, 50, 53, 54
- „ „ and *Trichoderma lignorum*, 174
- „ „ change to diploid phase, 186
- „ „ drops excreted by mycelium, 17
- „ „ fruit-body rudiments, 102-112
- „ „ geotropism, 117, 123
- „ „ heliotropism, 121-126
- „ „ homothallism, 155, 286
- „ „ hyphal fusions, 154, 157, 158, 159
- „ „ hyphal string in stipe, 72-73
- „ „ illustrations: three, 68-70; four, 79-83; sixteen, 95-122; seven, 156-164
- „ „ in competition with other fungi, 90-91
- „ „ ink of, 69-72
- „ „ mechanical fixation of fruit-body, 92-136

- Coprinus sterquilinus*, mycelium, experiment with, 90-91
- " " number of spores, 165
- " " oidia not produced by, 286
- " " relation of mycelium and fruit-body, 155-156
- " " social organisation in, 155-169
- " " spore germination, 78-91
- " " stipe-base, 112, 117
- " " stipe-shaft, 113
- " " upward pressure of stipe, 117-120
- " " water excretion, 74
- Coprinus* Type, 63-69
- Coprophilous Agaricineae, 85
- Craigie, J. H., on pycnidia of Rust fungi, 286
- " " on sexual process in Rust fungi, 281
- Criteria of sex, 198-203
- Cronartium ribicola*, spore-bed of, 283
- Cryptospora aucta*, hyphal fusions, 172
- Cudonia lutea*, ascogonia, 290
- Culture media, 85-86, 195-197
- Curtus Sub-type, and Atramentarius Sub-type, 2
- " " and Comatus Sub-type, 2, 4
- " " and Lagopus Sub-type, 2
- " " and Micaceus Sub-type, 2
- " " and Plicatilis Sub-type, 2, 3, 4
- " " description of, 1-4
- Cyanophyceae, social organisation, 140
- Cystidia of *Coprinus plicatilis*, 41, 45, 47, 54-55
- Cysts and cystophores of *Chondromyces crocatus*, 143-144, 148
- Cytology of young mycelia, 79-80, 83
- DAYLIGHT, effect on fruit-body development, 23-25, 49
- De Bary, A., on *Nectria Solani*, 172
- Dearness, J., and the fungi of Manitoba, 58
- Dictyostelium mucoroides*, illustration, 150
- Dictyostelium mucoroides*, social organisation, 144-146
- Didymium squamulosum*, cannibalism, 149
- Dinobryon, social organisation, 140
- Diploid mycelia, drawings of, 202, 271, 279, Plates III, IV
- " " effect on haploid mycelia, 187-268, 304, 305
- " " mated with haploid mycelia, first experiments, 204-213
- " " photograph of, 201
- " " two kinds of, 203-204
- Diploid mycelium, development of, 189
- " " effect of, compared with that of its two components, 253, 263
- " " simultaneous action on two haploid mycelia, 209-212, 303
- Diploidisation, and patchiness, 248, 249, 258
- " by a theoretically incompatible diploid, 245-263, 304-305
- " definition of, 190, 302
- " extent of, in a large mycelium, 229-231
- " in Uredineae, 281
- " incomplete, 212-213, 252, 303
- " of a haploid mycelium by a diploid, 187-268, 279, 280, 305, Plates III and IV
- " of a mycelial ring, 231-233
- " progressive, represented diagrammatically, 277, 280, Plates I-IV
- Diploidisation process, and conjugate nuclei, 278, 280
- " " progress of, in detail, 275-276
- Diploidise, definition of, 190, 302
- Diploidised hypha, further development of, 278
- Discomycetes, and conjugate nuclei, 188, 269, 289-291
- " social organisation in, 172-173, 177-178

- Doassansia, 287
 Dodge, B. O., on *Neurospora tetrasperma*, 290
 " " photograph by, 179
 Dodge, C. W., on *Coprinus comatus*, 74
 Douglas Fir, illustrations, 140, 141
 Dowding, E. Silver, on *Pleurage anserina*, 177, 290
 Drop-excretion, abnormal, from a sterigma, 31-32, 54
 " and spore-discharge, 31-32, 52-54
 " by hyphae, 17, 199
 Dry-needle method of sowing spores, 88, 194
 Duggar, B. M., culture method of, 86, 87
 Duke, Maud M., illustration by, 180
 " " on *Colletotrichum trichellum*, 179
 Dung-agar, how prepared, 195-197
 Durchbrechungskopulationen, 250, 251-252, 262, 263
 Dwarf fruit-bodies, 18, 23
 Eggs, no action of fertilised, on unfertilised, 264
 Entoloma, 287
 Eudorina, plasmodesmae, 143
 Evolution, of Coprini, 32
 " of groups of Basidiomycetes, 281, 287
 Exoascaceae, and conjugate nuclei, 188, 269, 288-289
 FALCK, R., on *Coprinus ephemerus*, 12
 " " on *Lenzites abietina*, 171
 Falck, R. and O., on *Psalliota campestris*, 169
 Field observations, on *Coprinus plicatilis*, 51-52
 Findlayson, R., and a *Coprinus* on Beet seeds, 74
Fistulina hepatica, water excretion, 74
 Fixation of fruit-bodies, conclusion, 133-136, 298-299
 " " discussion of factors concerned in, 126-133
 " " mechanical, 92-136
 Fixed position, importance of, 92, 126
Fomes applanatus, fruit-body fixation, 93
Fomes fomentarius, fruit-body fixation, 92-93
Fomes fomentarius, illustration, 93
Fomes officinalis, fruit-body fixation, 93
Fomes pinicola, kinds of spores, 270
 Frequency of conjugate nuclear divisions, 243, 304
 Fries, E., and *Coprinus curtus*, 9
 Fritsch, F. E., on *Eudorina*, 143
 " " on *Volvox*, 142
 Frost, and *Coprinus curtus*, 4-6
 Fruit-bodies, and compound mycelia, 163-169
 " diploid, 189
 " haploid, 189
 " haploid and diploid contrasted, 235, 265-266
 Fruit-body, and mycelium exhausted by
 " it, 155-156
 " fixed position of, 92, 126
 " function of, 68
 " origin of, 101, 164, 166, 167, 168
 Fruit-body efficiency, 64-69
 Fruit-body rudiments, 102-112
 " " and light, 104-112
 " " critical stage of development, 111
 " " of *Coprinus stercorearius*, 166, 167, 168
 " " supernumerary, 103
 Fruit-body size, in various Coprini, 32
 Fumes of fresh manure, effect on fruit-bodies, 9
 Fungi Imperfecti, hyphal fusions in, 178-179
 Fusion nucleus, diagram of, 272
 " " sex factors in, 203
Galera bulbifera, and *Chondromyces crocatus*, 143
Galera tenera, fixation of fruit-body, 135-136
 " " illustration, 135
 Gametes, absence of, in Basidiomycetes, 269, 281, 287
 Gardner, M. W., and a *Coprinus* on Beet seeds, 76
 Geotropic swinging, 26, 49
 Geotropism, of *Coprinus curtus*, 25
 " of *C. plicatilis*, 48-49
 " of *C. sterquilinus*, 117, 123

- Giant tramal cells, 20-21, 22, 28, 30
 Gill mass, 65
 Gills, ageotropic, 117
 „ of *Coprinus plicatilis*, non-deliquescent, 51
 „ powdered with spores, 56, 57
 „ splitting of, 20-21, 22, 27, 30, 44, 56
Gnomonia erythrostoma, ascogonia of, 290
Gnomonia leptostyla, sexual organs of, 290
 Gomphonema, social organisation, 140
Gomphosphaeria aponina, illustration, 142
 Gravity and fruit bodies, 25, 48-49, 117, 123
 Growth rate, of a mycelium of *Coprinus sterquilinus*, 91
 „ „ of haploid and diploid mycelia, 199
 „ „ of hyphae and rate of movement of nuclei, 223
- HANNA, W. F., and *Coprinus lagopus*, 190
 „ „ dry-needle method, 194
 „ „ on sex in *Coprinus lagopus*, 77, 193, 203
 „ „ on sex in *Ustilago*, 288
 „ „ on spores of *Coprinus sterquilinus*, 88-89
 „ „ on vigour of haploid and diploid fruit-bodies, 235
- Haploid and diploid fruit-bodies contrasted, 235
 Haploid and diploid mycelia, 154-155, 189, 199, 265
 Haploid chimaerae, 250
 Haploid fruit-bodies, feeble development of, 235
 Haploid hyphae, conversion into diploid, 237-241
 Haploid mycelia, drawings of, 196, 197, 198, 269
 „ „ effect of age on, 212-213, 303
 „ „ four kinds, 203-204
 „ „ fruiting of, 265, 267
 „ „ mutual diploidisation, of, 277
 „ „ photographs of, 195, 200
 Haploid mycelium, diploidised by a diploid, 204-213, 303
- Haploid mycelium, inoculated at centre, 207-209
 „ „ inoculated at periphery, 206-207
 „ „ movement of nuclei through, 213-229, 303-304
 Harder, R., on *Pholiota mutabilis*, 199
 Harper, R. A., on *Pyronema confluens*, 290
Hedera Helix, parasite on, 179
 Heliotropism, and geotropism, 123
 „ of *Coprinus curtus*, 25
 „ of *C. sterquilinus*, 121-126
Hemitrichia abietina, spore-like cells of, 149-150
 Heterothallic species of Hymenomycetes, 171, 184, 185
 Heterothallism of Hymenomycetes, 269-270
 Higher Fungi, 186
 Hive-bee, and *Coprinus sterquilinus*, 167-169, 301
 „ destruction of drones, 149
 Homothallic species of Hymenomycetes, 186
 Homothallism, in Uredineae, 286
 „ of *Coprinus sterquilinus*, 155
 Horses, and *Coprinus curtus*, 5.
 „ and *C. plicatilis*, 38
 „ and *C. sterquilinus*, 80-85, 165
Humaria granulata, ascogonia of, 290
 Hydathodes, cystidia as, 17-18
 Hydrodictyon, social organisation, 143
Hydrodictyon utriculatum, illustrations, 144, 145
 Hymenium, and gill mass, 65
 „ number of spores per unit area, 64
 „ of split gills, 56
 Hymenomycetes, and evolution, 32, 281, 287
 „ and Rust fungi, 281
 „ conclusion concerning nuclei of, 280-281
 „ heterothallic, 184-186
 „ homothallic, 186
 „ most species heterothallic, 171
 „ nuclei in diploid mycelium, 269
 „ nuclei of, 187-188
 „ rate of fall of spores, 48

- Hymenomycetes, rate of growth of mycelium, 199
 „ reduction divisions of nuclei, 252, 272
 „ significance of conjugate nuclei in, 269-291
 „ social organisation, 152-155
 „ violent spore - discharge, 53
 Hyphae, angle of branching, 198-199, 201-202
 „ conversion of haploid into diploid, 237-241
 „ lateral bulging of, 228
 „ on surface of culture media, 100, 198-199
 „ radial, number receiving nuclei, 241-242
 „ rate of growth, 91, 199, 223
 Hyphal fusions, and nuclei, 216
 „ „ and sex, 159, 184-186
 „ „ between diverse mycelia of same species, 184-186, 243-245, 304
 „ „ illustrations; twelve, 157-181; 196, 238, 246, 270, 271, 280, 283
 „ „ in *Coprinus lagopus*, 169-171
 „ „ in *C. stercorearius*, 169
 „ „ in *C. sterquilinus*, 157-169
 „ „ in homothallic Hymenomycetes, 186
 „ „ in Hymenomycetes, 152-155
 „ „ in *Panus stypticus*, 169
 „ „ in *Psalliota campestris*, 169
 „ „ in Rust fungi, 283
 „ „ in *Syncephalis*, 152
 „ „ number of, in a large mycelium, 158-159
 „ „ various functions of, 181-184
 Hyphal string, in stipes of Coprini, 72-73
Hypocrea rufa, hyphal fusions, 172, 173
Hypomyces rosellus, hyphal fusions, 172
 IDENTITY of each of two conjugate nuclei, 189
 Illegitimate combinations, haploid - diploid, 245-263, 302-303, 304-305
 „ „ haploid - haploid and haploid - diploid contrasted, 253, 254, 263
 Inaequi-hymeniferous Type, 63
 Incompatible combinations, haploid-diploid, 245-263, 302-303, 304-305
 Inhibition, of fusion of nuclei, 287, 291, 292
 „ of growth by light, 102-112
 Ink fungi, 69
 Inoculum, 207, 208, 215, 302
 Insects and oidia, 286
 JAHN, DR., on feeding of zygotes of Mycetozoa, 149
 Janet, E., on organisation of *Volvox*, 142-143
 KALCHBRENNER, C., on *Coprinus curtus*, 11
 Kew, 13, 38, 193
 Klebs, G., illustration by, 145
 „ „ on Hydrodictyon, 145
 Klein, L., on *Volvox globator*, 141
 Kniep, H., and *Coprinus fimetarius*, 193
 „ „ on clamp-connexions, 243
 „ „ on *Pyronema confluens*, 290
 „ „ on sex-factors and the mating of mycelia, 203
 „ „ on sex in *Ustilago*, 288
 „ „ on sexuality in Pyrenomycetes and Discomycetes, 290
 Knoll, F., on drops excreted by pileal hairs, 16, 17.
 LANGE, J. E., on *Coprinus curtus*, 11
 „ „ on *C. hemerobius*, 40
 Legitimate combinations, haploid-diploid, 204-213, 247, 302-304
 Lehfeldt, W., on nuclei, of *Typhula erythropus*, 216, 228
Lentinus lepideus, 120, 121
 Licmophora, social organisation, 140

- Licmophora flabellata*, illustration, 143
 Light, and periodic development of fruit-bodies, 23-25, 49
 „ and position of origin of fruit-bodies, 95
 „ heliotropic effect of, 121-126
 „ inhibitory effect on growth of fruit-body rudiments, 104-112
 „ morphogenic effect on fruit-bodies, 112-117
 Lister, G., and *Arcyria pomiformis*, 151
 „ „ on Mycetozoa, 147, 149
 Loeb, Jacques, on *Bryophyllum calycinum*, 103
 MACRAE, RUTH, as a research assistant, *vide* Preface, 175
 „ „ assisted with illustrations, 81, 83, 147, 159, 173, 175, 176, 180, 196, 202, 215, 222, 225, 233, 238, 246, 261
Marasmius oreades, and *Coprinus plicatilis*, 55
 „ „ heterothallic, 171
 „ „ pileus-flesh of, 66
Marasmius rotula, gill-collar, 42
 „ „ illustration of, 43
 Marquand, C. V. B., on upward pressure exerted by fungi, 120
 Massee, G., on *Coprinus plicatilis*, 55, 60
 Mating method of identifying a *Coprinus*, 77
 Mating of mycelia, equal, 205-206
 „ „ „ haploid - diploid - haploid, 209-212
 „ „ „ haploid - haploid and haploid - diploid under natural conditions, 264
 „ „ „ modes of, 204-212
 „ „ „ unequal, 206-209, 302
 Maximum rate of movement of nuclei, 219-226
 Mechanical fixation of fruit-bodies, 92-136
 Mechanical injuries of a mycelium, 182, 183-184
 Melampsora, and hyphal fusions, 283
Merulius lacrymans, surface mycelium of, 100
 „ „ water excretion by, 74
 Micro-dissection of hyphae, 199
 Monosporous mycelia, 195
 Morphogenic stimulus of light, 24, 49, 112-117
 Mounce, Irene, and *Coprinus lagopus*, 190
 „ „ on *Coprinus curtus*, 6
 „ „ on *C. lagopus*, 267
 „ „ on *C. sterquilinus*, 88
 „ „ on *Fomes pinicola*, 270
Mucor mucedo, illustration, 153
 „ „ no social organisation in, 152
 Mushrooms and Toadstools, 188
 Mutations, possibility of, 237, 251
 Mycelia, age and mating power, 212-213, 303
 „ and neuter bees, 167-168, 301
 „ competition and co-operation, 167
 „ compound, 163, 171-172, 174-177
 „ intermingling of, 282, 283
 „ kinds of haploid and diploid, 203-204
 „ monosporous, 195
 „ multinuclear nature of, and sex, 274
 „ $(n)+(n)$ and $(2n)$, 187-189, 268-269, 270, 278-281, 282, 288, 292, 305
 „ pairing of, 204-213
 „ social organisation of, 155-186
 Mycelial ring, diploidisation of, 231-233
 Mycelial strands, 101-102
 Mycelium, a three-dimensional network, 158
 „ at surface of substratum, 100, 198-199
 „ conduction of food materials through, 181, 182
 „ exhaustion of, 156, 165
 „ haploid contrasted with diploid, 198-199
 „ inoculation of a, 206-208
 „ just diploidised, experiment on, 229-231
 „ mass and fruiting capacity, 165
 „ mechanical injuries to, 182, 183-184
 „ movement of nuclei through, 181, 183, 213-229, 303-304
 „ network and movement of nuclei, 223

- Mycelium, of *Coprinus lagopus*, 170,
 198-204, 204-281
 „ of *C. stercorarius*, 166, 167,
 168
 „ of *C. sterquilinus*, 95-102,
 157-169
 „ possible nuclear fusion in,
 252
 „ rate of growth, 96, 199
 „ sex-factor analyses of, 234-
 237, 250-252
 „ sexual unions, 181, 182-183
 Mycetozoa, social organisation in, 147-
 152
 Myxobacteriaceae, and the Acrasieae,
 144
 „ social organisation
 in, 139, 143-144

Nectria Solani, illustration, 172
 „ „ social organisation, 172
Nectria Stilbosporae, hyphal fusions,
 172
Neurospora tetrasperma, sex of spores,
 290
 Newton, Dorothy E., and a *Coprinus*
 on Beet seeds,
 77
 „ „ and *Coprinus*
lagopus, 190
 „ „ illustration by,
 273
 „ „ on *Coprinus Ros-*
trupianus, 270
 „ „ on kinds of
 basidia, 273
 Nicrome wire loop, 195
 Non-*Coprinus* Type, 63-69
 Nuclear division, single and conjugate,
 189
 Nuclear divisions, number of, 242
 Nuclear fusion, in a basidium, 272
 „ „ in *Smut* fungi, 287
 Nuclear movement, an astonishing phe-
 nomenon, 228-229
 „ „ experiments on di-
 rection of, 229-
 234, 304
 „ „ in hyphae, cause of,
 228
 Nuclei, and breaking down of septa, 227-
 228
 „ and diploidisation process, 273-
 292, 305
 „ and hyphal fusions, 185-186, 216

 Nuclei, diagrammatic illustrations, 155,
 269-280, Plates I-IV
 „ fusion of, 272, 287, 305
 „ identity of, in a conjugate pair,
 280-281
 „ in haploid and diploid fruit-
 bodies, 266
 „ in homothallic Hymenomycetes,
 186
 „ in mycelia of *Coprinus sterqui-*
linus, 80, 83
 „ inequality of number and conju-
 gate nuclei, 291
 „ inhibited from fusing, 287, 291,
 292
 „ movement of, and hyphal fusions,
 181, 183
 „ movement of, in *Rust* fungi,
 283-284
 „ moving, direction taken by, 223,
 229-234, 304
 „ multiplication of, in a mycelium
 becoming diploidised, 242-243,
 304
 „ rate of movement and rate of
 growth of hyphae, 223
 „ rate of movement of, 189, 213-
 229, 303-304
 „ reduction divisions, 252
 „ width and movement, 226-227

 OIDIA, 198-199
 „ and pycnidiospores, 286
 „ borne on a haploid mycelium, 246
 „ illustrations of, 198, 200, 246
 „ of *Coprinus curtus*, 6
 „ of *C. lagopus*, 170, 198-199, 200,
 246
 Oidiophores, 198-199, 200, 246
 „ illustrations, 197, 198, 200,
 246
 Oltmanns, F., on *Volvox*, 142
 Oort, A. P. J., and *Coprinus fimetarius*,
 193
 „ „ on durchbrechungskopu-
 lationen, 250, 252
 Oxidase and *Coprinus* ink, 70
 Oxygen and *Coprinus* ink, 70

 PALISADE cells of pileus, 18, 38, 39, 43
 Panaeoli, and herbivorous animals, 85
Panaeolus campanulatus, and *Coprinus*
plicatilis, 54
 „ „ fixation, 134,
 135, 136

- Panaeolus campanulatus*, gelatinous mycelium, 101
 „ „ heterothallic, 171
 „ „ illustration, 134
Panaeolus Sub-type, 63
Panus stypticus, hyphal fusions, 152, 154, 169, 171
 Pape, and a *Coprinus* on Beet seeds, 75
 Paraphyses, as elastic elements in the hymenium, 28
 Parasitism of mycelia, 165
 Patchiness, phenomenon of, 248, 249
Parvulus involutus, fruit-body fixation, 93-94
 Periodicity in development of fruit-bodies, 23-25, 49, 52
 Petri dishes, examination of mycelia in, 203
 „ „ small and large, 196-197
 Phaeophyceae, social organisation in, 140
 Phanerogamia, no social organisation in, 139
 „ nuclei in diploid phase, 269
 „ union of nuclei, 188
 „ union of roots, 139, 140, 141
Pholiota mutabilis, rate of growth of mycelia, 199
Pholiota radicata, fruit-body fixation, 94
Phragmidium speciosum, spore-bed of, 283
Phragmidium violaceum, spore-bed of, 282
 Phycomycetes, and social organisation, 152
 Pileus-flesh, and fruit-body efficiency, 65-68
 „ divided into rays, 40, 41
 „ of *Coprinus comatus*, 73
 „ sheath of, 40
 „ volume of, 67
Pilobolus, drop-excretion from, 17
 „ and *Syncephalis*, 152
Pilobolus longipes, and fumes of fresh manure, 9
 „ „ nosocial organisation in, 152
Pilocystidia, illustrations of, 15, 16, 17, 18, 43
 „ of *Coprinus curtus*, 14-18
 „ of *C. plicatilis*, 43
 Plasmodesmae, 143
 Plasmodium, aggregate, 146
 Plasmodium, compound, 147
 „ formation of, 146, 147
Pleospora herbarum, hyphal fusions, 172
Pleurage anserina, bridging hyphae, 154
 „ „ illustrations, 177, 178
 „ „ sex of spores, 290
 „ „ social organisation, 177-178
Pleurocystidia, autodigestion of, 54
 „ of *Coprinus atramentarius*, 45
 „ of *C. lagopus*, 45
 „ of *C. plicatilis*, 45-46, 47
Plicatilis Sub-type, description, 33-35
 Pollock, J. B., culture medium of, 86
 Polyporaceae, fixation of fruit-bodies, 92-93
Polyporus squamosus, water excretion, 74
Polystigma rubrum, ascogonia of, 290
 Pressure, upward, exerted by a stipe, 117-120
Psalliota campestris, and *Coprinus comatus*, 66-68
 „ „ and *C. plicatilis*, 55
 „ „ fruit-body efficiency, 65-68
 „ „ fruit-body fixation, 93-94
 „ „ heliotropism, 125
 „ „ hyphal fusions, 169
 „ „ illustration, 66
 „ „ rate of fall of spores, 48
Psathyrella disseminata, and *Coprinus curtus*, 16
Psathyrella Sub-type, 63
Pseudorhiza, and fruit-body fixation, 94
 „ and the stipe-base, 112-113
Pseudotsuga taxifolia, illustrations, 140, 141
 Pteridophyta, no social organisation in, 139
 „ nuclei in diploid phase, 269
 „ union of nuclei, 188
Puccinia, species of, 283
Puccinia graminis, hyphal fusions, 283
 „ „ illustration, 283
 „ „ sexual process, 281, 286
Puccinia helianthi, sexual process, 281, 286
Puccinia Malvacearum, homothallism of, 286
Puccinia poarum, spore-bed of, 282

- Pycnidiospores, and oidia, 286
 " diploidisation by, 286
 Pyrenomycetes, and conjugate nuclei,
 188, 289-291
 " nuclei in ascogenous
 hyphae, 269
 " social organisation in,
 172-177
Pyronema confluens, sexual process of,
 290, 291-292

 RATE of fall of spores, 47-48
 Rate of growth of hyphae, 91, 199, 223
 Rate of movement of nuclei, 213-229,
 303-304
 Rea, Carleton, and *Coprinus pilosus*, 75
 Reduction divisions, of nuclei in my-
 celium, 252
 " " of nucleus in a
 basidium, 272
Rhizina undulata, ascogonia of, 290
 Roots, union of, in Douglas Fir, 140,
 141
 Rudiments of fruit-bodies, 101, 102-112
 Rust fungi, 281-287

Saprolegnia ferax, no social organisation
 in, 152
Schizophyllum commune, heterothallic,
 171
 " " kinds of spores,
 270
 Segregation of sex-factors, 272-274
 Septa, destruction of, during diploidi-
 sation process, 216, 227-228
 " in young mycelia, 80, 81
 Sex, and hyphal fusions in Hymeno-
 mycetes, 184-186
 " change to diploid phase in homo-
 thallic species, 186
 " compatibility, 204
 " criteria of, 198-203
 Sex-factor analysis of a mycelium, 234-
 237, 250-252
 Sex-factors, number of, and symbols for,
 203-204, 269-270
 " segregation of, 188, 272
 Sexual organs, absence of, 281, 287
 Seyfert, R., on clamp-connexions in Smut
 fungi, 287
 Smut fungi, 287-288
 Social organisation, general account, 139-
 186

 Social organisation, in animals and
 plants, 139
 " " of Ascomycetes, 172-
 178
 " " of *Coprinus sterqui-*
 linus, 152-169
 " " of other Hymeno-
 mycetes, 169-172
Sordaria fimiseda, hyphal fusions, 172
 Species, persistence of, and social or-
 ganisation, 168
 Specific gravity of spores, 48, 55
 Spore-deposit, how collected, 194
 " of *Coprinus plicatilis*, 55
 Spore-discharge, from a single basidium,
 53
 " hastening of, 31
 " in *Coprinus curtus*, 28-
 32
 " in *C. plicatilis*, 51-55
 " mode of demonstrating,
 31
 " zone of, 50
 Spore-like cells, of Mycetozoa, 151-152
 " " illustration of, 151
 Spores, always haploid, 270
 " and bacteria, 85-89, 90, 91
 " and *Coprinus* ink, 69-70, 72
 " and heat, 6, 89
 " and horses, 5, 38, 80-85
 " germination of, 78-91
 " many, in a dung-ball, 163-169
 " not easily destroyed, 72
 " number per unit area of hy-
 menium, 63-65
 " of *Coprinus lagopus*, four kinds,
 194-195
 " one in a dung-ball, 160-163
 " per unit volume of fruit-body, 67
 " photographed dry, 80
 " rate of fall of, 47-48
 " sexual kinds of, in heterothallic
 Hymenomycetes, 270
 " shape of, in various Coprini, 21,
 46-47
 " sown in unsterilised dung, 81-85
 " specific gravity of, 48, 55
 " trajectories of, 30
 " variability of, 39, 47-48
 Sprout-conidia, 288-289
 Sterigma, and drop-excretion, 31-32,
 54
 Sterile fruit-bodies, and fumes of fresh
 manure, 9
 Sterility, of certain mycelia, 167, 193

- Stipe, hyphal string in, 72-73
 „ length of, and width of pileus, 48
 „ upward pressure of, 117-120
 Stipe-base, definition of, 112
 „ effect of light on, 112-117
 Stipe-shaft, definition of, 113
 Stones, raised by fungi, 120-121
 Streeter, S. G., on heliotropism of stipes, 125
Stropharia semiglobata, and *Coprinus comatus*, 63-65
 „ „ fixation, 136
 „ „ hymenium and spores, 64
 „ „ illustration of, 64
 Strophariae, and herbivorous animals, 85
 Struggle for the substratum, 90-91, 96
 Summary, general, 295-305
 Supernumerary fruit-body rudiments, 103
 Syncephalis, hyphal fusions, 152
Taphrina epiphylla, life-history and sex, 288-289
Taphrina Klebahnii, life-history and sex, 288-289
 Temperature and spore-germination, 89
 Thallophyta, social organisation in, 139
 Thaxter, R., on *Chondromyces crocatus*, 143-144
Thelebolus stercorarius, ascogonia of, 290
 Tilletia, 287
 Tilletiaceae, and conjugate nuclei, 187-188, 287-288
Trichia decipiens, spore-like cells of, 149-150
Trichoderma lignorum, hyphal fusions, 173-177
 „ „ illustrations, 173, 175, 176
 „ „ social organisation in, 173-177
 Tubercinia, 287
 Tulasne, L.-R. and C., on hyphal fusions in *Pyrenomyces*, 172
 „ „ on *Hypocrea rufa*, 173
Typhula erythropus, nuclei of, 216, 218
 „ „ septa and nuclear movement in, 228
 UNEQUAL mating, 206-209, 215
 Uninucleate cells, artificially produced, 199
 Unsterilised horse dung, inoculated with mycelium, 90-91
 „ „ „ inoculated with spores, 81-85
 Uredineae, conjugate nuclei of, 187-188, 269, 281-287
 „ hyphal fusions in, 283
 „ hypotheses concerning sex in, 282-287
 Urocystis, 287
 Ustilaginaceae, and conjugate nuclei, 187-188, 269, 287-288
 Ustilago, 287
Ustilago avenae, sexual process, 287, 288, 289
Ustilago violacea, sexual process, 287, 288, 289
Ustilago zeae, sexual process, 287, 288
 VAN TIEGHEM, P. H., on *Dictyostelium*, 146
 Vanterpool, T. C., and *Coprinus plicatilis*, 37
Venturia inaequalis, sexual organs of, 290
Vermicularia trichella, hyphal fusions in, 179-181
 Volvocaceae, social organisation in, 140-143
 Volvox, and social organisation, 141-143
Volvox globator, organisation of, 141
 Voss, W., on hyphal fusions in *Rust* fungi, 283
 WATER, excreted by a pileus of *Coprinus niveus*, 74, 75
 „ excreted by hyphae, 17, 199
 „ excreted by spores, 31-32, 52-54
 Water-net, 140
 Wax pencil, use of, 195, 221
 West, G. S., illustrations by, 142
 Weston, W. A. Dillon, and a *Coprinus* on Beet seeds, 76
 Wieben, M., on sex in *Exoascaceae*, 289
 Wind, and spores of *Coprinus plicatilis*, 55, 57
 Woronin, M., on hyphal fusions in *Ascomycetes*, 172
 ZOFF, W., on *Syncephalis*, 152

